Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	0	WO200192523	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/07/18 09:12
L2	2	"200192523"	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/07/18 09:29
L3	202	phospholamban	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/07/18 09:29
L4	2	phospholamban.ab. and mutat?	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/07/18 09:30
L5	4	phospholamban and mutat?	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/07/18 09:31
L6	56	phospholamban and alanine	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/07/18 09:31

10 = 12-2-02

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File   5:Biosis Previews(R) 1969-2005/Jul W2
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Set     Items   Description
S1       1750   PHOSPHOLAMBAN
S2         66   MUTAT? AND S1
S3          9   FUSION AND S1
S4          0   FUSION AND S2
S5          8   S1 AND DELIVER?
S6         62   AU='KIMURA YOSHIHIRO'
S7         21   S1 AND S6
S8         16   AU='REED THOMAS D' OR AU='REED THOMAS'
S9          3   S1 AND S8

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DIALOG(R)File   5:Biosis Previews(R)
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0015313666   BIOSIS NO.: 200510008166
Rapid, high-yield expression and purification of Ca2+-ATPase regulatory
  proteins for high-resolution structural studies
AUTHOR: Douglas Jennifer L; Trieber Catharine A; Afara Michael; Young
  Howard S (Reprint)
AUTHOR ADDRESS: Univ Alberta, Dept Biochem, Edmonton, AB T6G 2H7, Canada**
  Canada
AUTHOR E-MAIL ADDRESS: hyoung@ualberta.ca
JOURNAL: Protein Expression and Purification  40 (1): p118-125 MAR 05 2005
ISSN: 1046-5928
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLB) and sarcolipin (SLN) are small integral
  membrane proteins that regulate the Ca2+-ATPases of cardiac and skeletal
  muscle, respectively, and directly alter their calcium transport
  properties. PLB interacts with and regulates the cardiac Ca2+-ATPase at
  submaximal calcium concentrations, thereby slowing relaxation rates and
  reducing contractility in the heart. SLN interacts with and regulates the
  skeletal muscle Ca2+-ATPase in a mechanism analogous to that used by PLB.
  While these regulatory interactions are biochemically and physiologically
  well characterized, structural details are lacking. To pursue structural
  studies, such as electron cryo-microscopy and X-ray crystallography,
  large quantities of over-expressed and purified protein are required.
  Herein, we report a modified method for producing large quantities of PLB
  and SLN in a rapid and efficient manner. Briefly, recombinant wild-type
  PLB and SLN were over-produced in Escherichia coli as maltose binding
  protein %%%fusion%%% proteins. A tobacco etch virus protease site allowed
  specific cleavage of the %%%fusion%%% protein and release of recombinant
  PLB or SLN. Selective solubilization with guanidine-hydrochloride
  followed by reverse-phase HPLC permitted the rapid, large-scale
  production of highly pure protein. Reconstitution and measurement of
  ATPase activity confirmed the functional interaction between our
  recombinant regulatory proteins and Ca2+-ATPase. The inhibitory
  properties of the over-produced proteins were consistent with previous
  studies, where the inhibition was relieved by elevated calcium
  concentrations. In addition, we show that our recombinant PLB and SLN are
  suitable for high-resolution structural studies. (c) 2004 Elsevier Inc.
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0013606026   BIOSIS NO.: 200200199537
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Reconstitution of the cytoplasmic interaction between %%%phospholamban%%% and Ca2+-ATPase of cardiac sarcoplasmic reticulum
AUTHOR: Kimura Yoshihiro; Inui Makoto (Reprint)
AUTHOR ADDRESS: Department of Pharmacology, Yamaguchi University School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi, 755-8505, Japan**Japan
JOURNAL: Molecular Pharmacology 61 (3): p667-673 March, 2002 2002
MEDIUM: print
ISSN: 0026-895X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLN) reversibly inhibits the Ca2+-ATPase of cardiac sarcoplasmic reticulum (SERCA2a) through a direct protein-protein interaction, playing a pivotal role in the regulation of intracellular Ca2+ in heart muscle cells. The interaction between PLN and SERCA2a occurs at multiple sites within the cytoplasmic and membrane domains. Here, we have reconstituted the cytoplasmic protein-protein interaction using bacterially expressed %%%fusion%%% proteins of the cytoplasmic domain of PLN and the long cytoplasmic loop of SERCA2a. We have developed two methods to evaluate the binding of the %%%fusion%%% proteins, one with glutathione-Sepharose beads and the other with a 96-well plate. Essentially the same results were obtained by the two methods. The affinity of the binding (KD) was 0.70 muM. The association was inhibited by cAMP-dependent phosphorylation of the PLN %%%fusion%%% protein and by usage of anti-PLN monoclonal antibody. It was also diminished by substitution at the phosphorylation site of PLN of Ser16 to Asp. These results suggest that PLN can bind SERCA2a in the absence of the membrane domains and that the modifications of the cytoplasmic domain of PLN that activate SERCA2a parallel the disruption of the association between the two %%%fusion%%% proteins. It has been shown that the removal of PLN inhibition of SERCA2a rescues cardiac function and morphology in the mouse dilated cardiomyopathy model. Our assay system can be applied to the screening of novel inotropic agents that remove the inhibition of SERCA2a by PLN, improving the relaxation as well as the contractility of the failing heart.

3/7/3
DIALOG(R)File 5:Biosis Previews(R)

0012586507 BIOSIS NO.: 200000304820
Soluble tumor necrosis factor receptor abrogates myocardial inflammation but not hypertrophy in cytokine-induced cardiomyopathy
AUTHOR: Kubota Toru; Bounoutas George S; Miyagishima Masayuki; Kadokami Toshiaki; Sanders Virginia J; Bruton Christina; Robbins Paul D; McTiernan Charles F; Feldman Arthur M (Reprint)
AUTHOR ADDRESS: 200 Lothrop St, S 572 Scaife Hall, Pittsburgh, PA, 15213, USA**USA
JOURNAL: Circulation 101 (21): p2518-2525 May 30, 2000 2000
MEDIUM: print
ISSN: 0009-7322
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Background-Transgenic mice with cardiac-specific overexpression of tumor necrosis factor (TNF)-alpha develop dilated cardiomyopathy. The present study was designed to evaluate therapeutic effects of adenovirus-mediated neutralization of TNF-alpha on this model. Methods and Results-An adenovirus encoding the 55-kDa TNF receptor-IgG %%%fusion%%% protein (AdTNFRI) was injected intravenously into 6-week-old transgenic mice, which resulted in high levels of TNFRI in both plasma and myocardium. AdTNFRI did not reverse cardiomegaly but abrogated myocardial inflammation. Furthermore, AdTNFRI blocked the myocardial expression of intercellular adhesion molecule-1 and downstream cytokines, including interleukin-1beta and monocyte chemotactic protein-1. Downregulation of alpha-myosin heavy chain was restored by the treatment, whereas upregulation of beta-myosin heavy chain was not reversed. In contrast, the downregulation of sarcoplasmic reticulum Ca2+ -ATPase and

%%%phospholamban%%% was normalized by AdTNFRI. Echocardiographic measurements showed that left ventricular end-systolic diameter was significantly larger in transgenic mice than in control mice, and this increase was reversed by the AdTNFRI treatment. However, left ventricular wall thickening was not reversed. Conclusions-These results suggest that anti-TNF therapy may hold promise in the treatment of end-stage heart failure.

3/7/4
DIALOG(R)File 5:Biosis Previews(R)

0011253113 BIOSIS NO.: 199800047360
In vitro and in vivo promoter analyses of the mouse %%%phospholamban%%% gene
AUTHOR: Haghighi Kobra; Kadambi Vivek J; Koss Kimberly L; Luo Wusheng; Harrer Judy M; Ponniah Sathivel; Zhou Zuoping; Kranias Evangelia G (Reprint)
AUTHOR ADDRESS: Dep. Pharmacol. Cell Biophysics, Univ. Cincinnati, Coll. Med., 231 Bethesda Ave., Cincinnati, OH 45267-0575, USA**USA
JOURNAL: Gene (Amsterdam) 203 (2): p199-207 Dec. 12, 1997 1997
MEDIUM: print
ISSN: 0378-1119
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: To determine the mechanisms responsible for regulation of the %%%phospholamban%%% (PLB) gene expression, a critical regulatory phosphoprotein in cardiac muscle, the mouse PLB gene was isolated and promoter analysis was performed in vitro and in vivo. The PLB gene consists of two exons separated by a single large intron. Deletion analysis revealed that a 7-kb 5' flanking fragment (including exon 1, the entire intron and part of exon 2) was necessary for maximal transcriptional activity in H9c2 and L6 cell lines. Interestingly, deletion of a 2.4-kb intronic region, which contained repetitive elements, caused a dramatic increase in CAT activity in both these cell lines. In vivo analysis indicated that the PLB %%%fusion%%% gene containing 7 kb of the 5'-flanking region was capable of cardiac specific gene expression in transgenic mice. Furthermore, these mice exhibited 3-fold higher levels of CAT activity in the ventricles compared with the atria, mimicking endogenous PLB mRNA expression. Our findings suggest that: (a) PLB gene expression may be regulated by the interplay of cis-acting regulatory elements located within the 5' flanking and intronic regions; and (b) the 7-kb upstream region is capable of directing cardiac-specific and compartment-specific expression in vivo.

3/7/5
DIALOG(R)File 5:Biosis Previews(R)

0011161685 BIOSIS NO.: 199799795745
Purification of the cardiac sarcoplasmic reticulum membrane protein %%%phospholamban%%% from recombinant Escherichia coli
AUTHOR: Kromer Wolfgang J; Carafoli Ernesto; Bailey James E (Reprint)
AUTHOR ADDRESS: Inst. Biotechnol., ETH Zurich, CH-8093 Zurich, Switzerland **Switzerland
JOURNAL: European Journal of Biochemistry 248 (3): p814-819 1997 1997
ISSN: 0014-2956
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLN) was expressed in Escherichia coli as a protein %%%fusion%%% with glutathione S-transferase (GST). GST-PLN was mostly present in the insoluble protein fraction and accounted for approximately 50% of total insoluble protein. Attempts to suppress inclusion body formation or to use GST as an affinity-purification tag failed. A successful purification method is based on preparative SDS/PAGE

7P w/ PLN

and electrodialysis. From 1 g cells we typically purified 13.5 mg %%%fusion%%% protein with a PLN content of 2.8 mg. We genetically inserted an enterokinase (EK) protease site just in front of the PLN sequence and demonstrated the proteolytical liberation of PLN from the carrier protein. The approach described represents a substantial advancement in PLN expression and purification.

3/7/6
DIALOG(R)File 5:Biosis Previews(R)

0010695773 BIOSIS NO.: 199799329833
Purification of porcine %%%phospholamban%%% expressed in Escherichia coli
AUTHOR: Yao Qing; Bevan Judy L; Weaver Robert F; Bigelow Diana J (Reprint)
AUTHOR ADDRESS: Dep. Biochemistry, Univ. Kansas, Lawrence, KS 66045, USA** USA
JOURNAL: Protein Expression and Purification 8 (4): p463-468 1996 1996
ISSN: 1046-5928
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLB) is a small hydrophobic protein that regulates contractility in the heart. This membrane protein expressed in bacterial cells is resistant to purification by conventional strategies that have been used to isolate expressed soluble proteins. Therefore, in order to obtain both wild-type and mutant PLB proteins, we have amplified the PLB gene by the polymerase chain reaction from genomic DNA of porcine heart and inserted it into the pGEX-2T plasmid expression vector. In this vector, the gene product fused to glutathione S-transferase has been expressed in JM109 Escherichia coli cells. The expressed %%%fusion%%% protein was found associated predominantly with insoluble cellular constituents. However, most of the %%%fusion%%% protein was readily extracted with SDS. PLB was subsequently purified by a simple procedure consisting of isolation of the %%%fusion%%% protein by preparative SDS-gel electrophoresis, followed by a second electrophoretic separation of PLB after its cleavage from the %%%fusion%%% protein by thrombin. This isolation method yields 3-4 mg of PLB per liter of cells, in a form which is capable of functional interaction with the Ca-ATPase in reconstituted proteoliposomes.

3/7/7
DIALOG(R)File 5:Biosis Previews(R)

0010614882 BIOSIS NO.: 199699248942
Salbutamol and chronic low-frequency stimulation of canine skeletal muscle
AUTHOR: Hu Ping; Zhang Ke-Min; Feher Joseph J; Wang Shang-Wu; Wright Leon D ; Wechsler Andrew S; Spratt John A; Briggs F Norman (Reprint)
AUTHOR ADDRESS: Dep. Physiol., Med. Coll. Virginia, Virginia Commonwealth Univ., Richmond, VA 23298-0551, USA**USA
JOURNAL: Journal of Physiology (Cambridge) 496 (1): p221-227 1996 1996
ISSN: 0022-3751
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: 1. The effect of simultaneous application of chronic muscle stimulation and salbutamol on the expression of mRNAs and proteins normally expressed by fast- or slow-twitch fibres was followed and the effects of changes in protein expression on mechanical performance were evaluated. Chronic low-frequency stimulation increased the myosin heavy chain (HC)-I level in the canine latissimus dorsi muscle and simultaneous administration of salbutamol partially blocked this change. Associated with the increase in HC-I level was a decrease in the velocity of shortening at zero load, V-max. The change in V-max was partially blocked by salbutamol. 2. Chronic low-frequency stimulation increased the levels of slow-twitch cardiac isoform sarco-/endoplasmic reticulum Ca-2+-ATPase SERCA2a and %%%phospholamban%%% mRNA and SERCA2a and %%%phospholamban%%%

protein expression. These changes were associated with an increase in time-to-peak tension and a decrease in %%%fusion%%% frequency. Simultaneous administration of salbutamol blocked these changes in protein expression and muscle mechanics. Chronic stimulation of latissimus dorsi decreased the levels of the fast-twitch isoform of sarco-/endoplasmic reticulum Ca-2+-ATPase (SERCA1a) and increased SERCA2a protein expression and decreased calcium uptake rate by muscle homogenates. These changes were blocked by salbutamol. 3. The loss of latissimus dorsi muscle weight by chronic stimulation was partially blocked by salbutamol.

3/7/8
DIALOG(R)File 5:Biosis Previews(R)

0010614881 BIOSIS NO.: 199699248941
Salbutamol changes the molecular and mechanical properties of canine skeletal muscle
AUTHOR: Zhang Ke-Min; Hu Ping; Wang Shang-Wu; Feher Joseph J; Wright Leon D ; Wechsler Andrew S; Spratt John A; Briggs F Norman (Reprint)
AUTHOR ADDRESS: Dep. Physiol., Med. Coll. Virginia, Virginia Commonwealth Univ., Richmond, VA 23298-0551, USA**USA
JOURNAL: Journal of Physiology (Cambridge) 496 (1): p211-220 1996 1996
ISSN: 0022-3751
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: 1. Salbutamol, a beta-2-agonist, increased the weight of the canine latissimus dorsi muscle. It also increased %%%fusion%%% frequency, and decreased time-to-peak tension, half-relaxation time, and total contraction time. These changes in twitch times and %%%fusion%%% frequency were associated with changes in the levels of proteins expressed in slow- and fast-twitch fibres. Salbutamol decreased the levels of the slow-twitch cardiac isoform of sarco-/endoplasmic reticulum Ca-2+ATPase (SERCA2a) and %%%phospholamban%%% proteins, and increased the level of the fast-twitch isoform of sarco-/endoplasmic reticulum Ca-2+-ATPase (SERCA1a). 2. Changes in the levels of SERCA proteins, particularly SERCA1a, could account for most of the increases in calcium uptake rate observed in homogenates of muscles from the salbutamol-treated animals and could partially account for the changes in half-relaxation rates and other twitch times. 3. Changes in the levels of SERCA1a, SERCA2a and %%%phospholamban%%% protein did not always follow changes in the levels of their corresponding mRNAs. Divergence depended upon the SERCA isoform and muscle. The muscles studied were latissimus dorsi and vastus intermedius. 4. Salbutamol did not change the level of myosin heavy chain (HC)-I isoforms in either muscle, suggesting that it did not increase the proportion of slow-twitch fibres in these muscles. It did increase the level of HC-IIx and decrease the level of HC-IIa isoforms in the latissimus dorsi. Salbutamol did not produce these effects in the vastus intermedius. It is of particular interest that salbutamol changed the relative levels of SERCA proteins in the latissimus dorsi muscle without producing significant changes in the level of HC-I isoform.

3/7/9
DIALOG(R)File 5:Biosis Previews(R)

0007172806 BIOSIS NO.: 199089090697
THE EXPRESSION OF CANINE CARDIAC %%%PHOSPHOLAMBAN%%% IN HETEROLOGOUS SYSTEMS
AUTHOR: COOK E A (Reprint); HUGGINS J P; SATHE G; ENGLAND P J; PIGGOTT J R
AUTHOR ADDRESS: DEP CELL PHARMACOL, SMITH KLINE FRENCH RES LTD, FRYTHE, WELWYN, HERTS AL6 9AR, UK**UK
JOURNAL: Biochemical Journal 264 (2): p533-538 1989
ISSN: 0264-6021
DOCUMENT TYPE: Article
RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: A synthetic %%%phospholamban%%% gene has been cloned and expressed in Escherichia coli, producing both native %%%phospholamban%%% and a %%%fusion%%% protein with 81 amino acids of the influenza virus NS1 protein. Both the native %%%phospholamban%%% and %%%fusion%%% proteins produced extensive cell lysis upon induction of gene expression, but only the native protein underwent spontaneous pentamer formation in E. coli. Translation in vitro of mRNA produced by transcription in vitro of %%%phospholamban%%% cDNA was used to demonstrate the spontaneous aggregation of %%%phospholamban%%% to form pentamers in this system also, both in the presence and absence of exogenous microsomes from canine pancreas or heart. %%%Phospholamban%%% produced by translation in vitro was apparently susceptible to proteolysis by enzymes present in the particulate fractions in these experiments.

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Set     Items   Description
S1       1750   PHOSPHOLAMBAN
S2         66   MUTAT? AND S1
S3          9   FUSION AND S1
S4          0   FUSION AND S2
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            1750  S1
          166753  DELIVER?
      S5       8  S1 AND DELIVER?
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DIALOG(R)File   5:Biosis Previews(R)
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0015353199   BIOSIS NO.: 200510047699
Ouabain upregulates phosphatase inhibitor-1 expression and sensitizes rat
  hearts to beta-adrenergic stimulation
AUTHOR: El-Armouche Ali (Reprint); Jaeckel Elmar; Eschenhagen Thomas
AUTHOR ADDRESS: Med Sch, Dept Gastroenterol, Hannover, Germany**Germany
JOURNAL: Journal of Molecular and Cellular Cardiology  36 (5): p727 MAY 04
2004
CONFERENCE/MEETING: 24th Annual Scientific Sessions of the European-Section
of the International-Society-for-Heart-Research  Dresden, GERMANY  June 02
-06, 2004; 20040602
SPONSOR: Int Soc Heart Res, European Sect
ISSN: 0022-2828
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English


 5/7/2
DIALOG(R)File   5:Biosis Previews(R)
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0015350465   BIOSIS NO.: 200510044965
Enhancement of cardiac function and suppression of heart failure
  progression by inhibition of protein phosphatase 1
AUTHOR: Pathak Anand; del Monte Federica; Zhao Wen; Schultz Jo-El; Lorenz
  John N; Bodi Ilona; Weiser Doug; Hahn Harvey; Carr Andrew N; Syed Faisal;
  Mavila Nirmala; Jha Leena; Qian Jiang; Marreez Yehia; Chen Guoli; McGraw
  Dennis W; Heist E Kevin; Guerrero J Luis; DePaoli-Roach Anna A; Hajjar
  Roger J; Kranias Evangelia G (Reprint)
AUTHOR ADDRESS: Univ Cincinnati, Coll Med, Dept Pharmacol and Cell Biophys,
  231 Albert Sabin Way,POB 670575, Cincinnati, OH 45267 USA**USA
AUTHOR E-MAIL ADDRESS: Litsa.Kranias@uc.edu
JOURNAL: Circulation Research  96 (7): p756-766 APR 15 05 2005
ISSN: 0009-7330
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Abnormal calcium cycling, characteristic of experimental and
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human heart failure, is associated with impaired sarcoplasmic reticulum calcium uptake activity. This reflects decreases in the cAMP-pathway signaling and increases in type 1 phosphatase activity. The increased protein phosphatase 1 activity is partially due to dephosphorylation and inactivation of its inhibitor-1, promoting dephosphorylation of %%%phospholamban%%% and inhibition of the sarcoplasmic reticulum calcium-pump. Indeed, cardiac-specific expression of a constitutively active inhibitor-1 results in selective enhancement of %%%phospholamban%%% phosphorylation and augmented cardiac contractility at the cellular and intact animal levels. Furthermore, the beta-adrenergic response is enhanced in the transgenic hearts compared with wild types. On aortic constriction, the hypercontractile cardiac function is maintained, hypertrophy is attenuated and there is no decompensation in the transgenics compared with wild-type controls. Notably, acute adenoviral gene %%%delivery%%% of the active inhibitor-1, completely restores function and partially reverses remodeling, including normalization of the hyperactivated p38, in the setting of pre-existing heart failure. Thus, the inhibitor 1 of the type 1 phosphatase may represent an attractive new therapeutic target.

5/7/3
DIALOG(R)File 5:Biosis Previews(R)

0015261659 BIOSIS NO.: 200500168395
Rescue of Ca2+ overload-induced left ventricle dysfunction by targeted ablation of %%%phospholamban%%%
AUTHOR: Tsuji Tsuyoshi; Del Monte Federica; Yoshikawa Yoshiro; Abe Takehisa ; Taniguchi Shigeki; Takaki Miyako; Hajjar Roger J
JOURNAL: Journal of Molecular and Cellular Cardiology 37 (5): p1084 November 2004 2004
MEDIUM: print
CONFERENCE/MEETING: 21st Annual Meeting of the Japanese Section of the International Society for Heart Research (ISHR) Kofu City, Yamanashi, Japan November 23-25, 2004; 20041123
SPONSOR: International Society for Heart Research
ISSN: 0022-2828 _(ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

5/7/4
DIALOG(R)File 5:Biosis Previews(R)

0014801385 BIOSIS NO.: 200400172142
Chronic %%%phospholamban%%% inhibition prevents progressive cardiac dysfunction and pathological remodeling after infarction in rats.
AUTHOR: Iwanaga Yoshitaka; Hoshijima Masahiko; Gu Yusu; Iwatate Mitsuo; Dieterle Thomas; Ikeda Yasuhiro; Date Moto-o; Chrast Jacqueline; Matsuzaki Masunori; Peterson Kirk L; Chien Kenneth R; Ross John (Reprint)
AUTHOR ADDRESS: Department of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA, 92093-0613, USA**USA
AUTHOR E-MAIL ADDRESS: jross@ucsd.edu
JOURNAL: Journal of Clinical Investigation 113 (5): p727-736 March 2004 2004
MEDIUM: print
ISSN: 0021-9738
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Ablation or inhibition of %%%phospholamban%%% (PLN) has favorable effects in several genetic murine dilated cardiomyopathies, and we showed previously that a pseudophosphorylated form of PLN mutant (S16EPLN) successfully prevented progressive heart failure in cardiomyopathic hamsters. In this study, the effects of PLN inhibition were examined in rats with heart failure after myocardial infarction (MI), a model of acquired disease. S16EPLN was %%%delivered%%% into failing hearts 5 weeks

112. treatment?

after MI by transcoronary gene transfer using a recombinant adeno-associated virus (rAAV) vector. In treated (MI-S16EPLN, n=16) and control (MI-saline, n=18) groups, infarct sizes were closely matched and the left ventricle was similarly depressed and dilated before gene transfer. At 2 and 6 months after gene transfer, MI-S16EPLN rats showed an increase in left ventricular (LV) ejection fraction and a much smaller rise in LV end-diastolic volume, compared with progressive deterioration of LV size and function in MI-saline rats. Hemodynamic measurements at 6 months showed lower LV end-diastolic pressures, with enhanced LV function (contractility and relaxation), lowered LV mass and myocyte size, and less fibrosis in MI-S16EPLN rats. Thus, PLN inhibition by in vivo rAAV gene transfer is an effective strategy for the chronic treatment of an acquired form of established heart failure.

5/7/5
DIALOG(R)File 5:Biosis Previews(R)

0013848589 BIOSIS NO.: 200200442100
Chronic suppression of heart-failure progression by a pseudophosphorylated mutant of %%%phospholamban%%% via in vivo cardiac rAAV gene %%%delivery%%%
AUTHOR: Hoshijima Masahiko; Ikeda Yasuhiro; Iwanaga Yoshitaka; Minamisawa Susumu; Date Moto-o; Gu Yusu; Iwatate Mitsuo; Li Manxiang; Wang Lili; Wilson James M; Wang Yibin; Ross John Jr; Chien Kenneth R (Reprint)
AUTHOR ADDRESS: University of California, San Diego (UCSD) Institute of Molecular Medicine, La Jolla, CA, USA**USA
JOURNAL: Nature Medicine 8 (8): p864-871 August, 2002 2002
MEDIUM: print
ISSN: 1078-8956
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The feasibility of gene therapy for cardiomyopathy, heart failure and other chronic cardiac muscle diseases is so far unproven. Here, we developed an in vivo recombinant adeno-associated virus (rAAV) transcoronary %%%delivery%%% system that allows stable, high efficiency and relatively cardiac-selective gene expression. We used rAAV to express a pseudophosphorylated mutant of human %%%phospholamban%%% (PLN), a key regulator of cardiac sarcoplasmic reticulum (SR) Ca2+ cycling in BIO14.6 cardiomyopathic hamsters. The rAAV/S16EPLN treatment enhanced myocardial SR Ca2+ uptake and suppressed progressive impairment of left ventricular (LV) systolic function and contractility for 28-30 weeks, thereby protecting cardiac myocytes from cytopathic plasma-membrane disruption. Low LV systolic pressure and deterioration in LV relaxation were also largely prevented by rAAV/S16EPLN treatment. Thus, transcoronary gene transfer of S16EPLN via rAAV vector is a potential therapy for progressive dilated cardiomyopathy and associated heart failure.

5/7/6
DIALOG(R)File 5:Biosis Previews(R)

0012234865 BIOSIS NO.: 199900494525
Calcium regulatory proteins and their alteration by transgenic approaches
AUTHOR: Dillmann W H (Reprint)
AUTHOR ADDRESS: Endocrinology and Metabolism, University of California San Diego, 9500 Gilman Drive (BSB 1 5063), La Jolla, CA, 92093-0618, USA**USA
JOURNAL: American Journal of Cardiology 83 (12A): p89H-91H June 17, 1999 1999
MEDIUM: print
ISSN: 0002-9149
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Abnormalities in calcium flux have been linked to abnormal contractile behavior of the heart in patients with congestive heart

failure as well as in animal models. Decreased activity or levels of the calcium adenosine triphosphatase of the sarco(endo)plasmic reticulum (SERCA2) particularly have been known to cause a delay in calcium transients. The SERCA2 protein pumps 2 moles of calcium per mole of adenosine triphosphate (ATP) split from the cytoplasm into the sarcoplasmic reticulum, thus lowering the free cytoplasmic calcium concentration. It therefore is of interest to identify mechanisms by which SERCA activity could be increased in the heart. To determine influences of increased expression of the SERCA2 gene on calcium transient and contractile behavior, we constructed transgenic mice and rats expressing a SERCA2 transgene in their heart. In these animals, a 20% increase in SERCA levels occurs due to additional expression of the SERCA transgene. This leads to a corresponding increase in contractile activity as determined by the increase in left ventricular pressure measured as dP/dtmax and decrease in diastolic ventricular pressure determined as dP/dtmin. Similarly, isolated cardiac myocytes obtained from the heart of transgenic mice showed an accelerated calcium transient and increased speed of shortening and relengthening as determined by edge detection. To determine if SERCA2 transgene expression could have a compensatory effect on the contractile behavior of the heart in transgenic mice expressing SERCA2, these mice were made hypothyroid, and papillary muscle function was determined. Contractile behavior of the papillary muscle of wild-type hypothyroid mice showed a significant increase in muscle relaxation time (RT50). In contrast, SERCA2 transgenic hypothyroid mice showed normal contractile behavior of papillary muscle. A compensatory effect of SERCA transgene expression was therefore demonstrated. In addition, we constructed transgenic rats expressing a SERCA2 transgene in which constriction of the ascending aorta induced cardiac hypertrophy and a delayed contraction of papillary muscle. In preliminary results, we found that SERCA2 transgenic rats submitted to ascending aortic constriction did not show the delayed relaxation of papillary muscle as was found in wild-type rats submitted to aortic constriction. In addition, adenoviral vectors expressing transgenes for calcium-handling proteins can be used to improve cardiac myocyte contraction. Adenoviruses expressing a SERCA transgene or a mutant %%%phospholamban%%% transgene exhibiting dominant negative action were used to infect isolated myocytes treated with a phorbol ester (phorbol 12-myristate 13-acetate), which delays the calcium transients. The calcium transients and contractile behavior of the isolated myocytes indicated that increased SERCA expression or increased expression of mutant %%%phospholambah%%% transgene led to increased SERCA2 activity, resulting in an increased contractile phenotype. Recent findings by other investigators also indicate that decreased SERCA2 activity can be increased under in vivo conditions using adenoviral vector-based SERCA2 expression. A gene therapy type of approach %%%delivering%%% increased amounts of SERCA or %%%phospholamban%%% mutants leading to increased SERCA activity should therefore be considered in the future.

5/7/7
DIALOG(R)File 5:Biosis Previews(R)

0010271634 BIOSIS NO.: 199698739467
Arterial %%%delivery%%% of genetically labelled skeletal myoblasts to the murine heart: Long-term survival and phenotypic modification of implanted myoblasts
AUTHOR: Robinson Shawn W; Cho Peter W; Levitsky Hyam I; Olson Jean J; Hruban Ralph H; Acker Michael A; Kessler Paul D (Reprint)
AUTHOR ADDRESS: Johns Hopkins Univ. Sch. Med., Ross Res. Room 812, 720 Rutland Ave., Baltimore, MD 21205, USA**USA
JOURNAL: Cell Transplantation 5 (1): p77-91 1996 1996
ISSN: 0963-6897
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The ability to replace damaged myocardial tissue with new striated muscle would constitute a major advance in the treatment of diseases that irreversibly injure cardiac muscle cells. The creation of focal grafts of skeletal muscle has been reported following the

intramural injection of skeletal myoblasts into both normal and injured myocardium. The goals of this study were to determine whether skeletal myoblast-derived cells can be engrafted into the murine heart following arterial %%%delivery%%%. The murine heart was seeded with genetically labeled C2C12 myoblasts introduced into the arterial circulation of the heart via a transventricular injection. A transventricular injection provided access to the coronary and systemic circulations. Implanted cells were characterized using histochemical staining for beta-galactosidase, immunofluorescent staining for muscle-specific antigens, and electron microscopy. Initially the injected cells were observed entrapped in myocardial capillaries. One week after injection myoblasts were present in the myocardial interstitium and were largely absent from the myocardial capillary bed. Implanted cells underwent myogenic development, characterized by the expression of a fast-twitch skeletal muscle sarcoplasmic reticulum calcium ATPase (SERCA1) and formation of myofilaments. Four months following injection myoblast-derived cells began to express a slow-twitch/cardiac protein, %%%phospholamban%%%, that is normally not expressed by C2C12 cells in vitro. Most surprisingly, regions of close apposition between LacZ labeled ells and native cardiomyocytes contained structures that resembled demosomes, fascia adherens junctions, and gap junctions. The cardiac gap junction protein, connexin43, was localized to some of the interfaces between implanted cells and cardiomyocytes. Collectively, these findings suggest that arterially %%%delivered%%% myoblasts can be engrafted into the heart, and that prolonged residence in the myocardium may alter the phenotypes of these skeletal muscle-derived cells. Furhter studies are necessary to determine whether arterial %%%delivery%%% of skeletal myoblasts can be developed as treatment for myocardial dysfunction.

5/7/8
DIALOG(R)File 5:Biosis Previews(R)

0009319021 BIOSIS NO.: 199497340306
Arterial %%%delivery%%% of skeletal myoblasts to the heart
AUTHOR: Robinson Shawn W (Reprint); Cho Peter C (Reprint); Acker Michael A; Kessler Paul D (Reprint)
AUTHOR ADDRESS: Dep. Med., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205, USA**USA
JOURNAL: Journal of Cellular Biochemistry Supplement 0 (18D): p531 1994 1994
CONFERENCE/MEETING: Keystone Symposium on Molecular Biology of Muscle Development Snowbird, Utah, USA April 11-17, 1994; 19940411
ISSN: 0733-1959
DOCUMENT TYPE: Meeting; Meeting Abstract; Meeting Poster
RECORD TYPE: Citation
LANGUAGE: English

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2/7/1
DIALOG(R)File 5:Biosis Previews(R)

0015343817 BIOSIS NO.: 200510038317
Role of leucine 31 of %%%phospholamban%%% in structural and functional interactions with the Ca2+ pump of cardiac sarcoplasmic reticulum
AUTHOR: Chen Zhenhui (Reprint); Stokes David L; Jones Larry R
AUTHOR ADDRESS: Krannert Inst Cardiol, 1800 N Capitol Ave, Indianapolis, IN 46202 USA**USA
AUTHOR E-MAIL ADDRESS: lrjones@iupui.edu
JOURNAL: Journal of Biological Chemistry 280 (11): p10530-10539 MAR 18 05 2005
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The ability of two loss-of-function mutants, L31A and L31C, of %%%phospholamban%%% (PLB) to bind to and inhibit the Ca2+ pump of cardiac sarcoplasmic reticulum (SERCA2a) was investigated using a molecular cross-linking approach. Leu(31) of PLB, located at the cytoplasmic membrane boundary, is a critical amino acid shown previously to be essential for Ca2+-ATPase inhibition. We observed that L31A or L31C %%%mutations%%% of PLB prevented the inhibition of Ca2+-ATPase activity and disabled the cross-linking of N27C and N30C of PLB to Lys(328) and Cys(318) of SERCA2a. Although L31C-PLB failed to cross-link to any Cys or Lys residue of wild-type SERCA2a, L31C did cross-link with high efficiency to T317C of SERCA2a with use of the homobifunctional sulfhydryl cross-linking reagent, 1,6-bismaleimidohexane. This places Leu(31) of PLB within 10 angstrom of Thr(317) of SERCA2a in the M4 helix. Thus, contrary to previous suggestions, PLB with loss-of-function %%%mutations%%% at Leu(31) retains the ability to bind to SERCA2a, despite losing inhibitory activity. Cross-linking of L31C-PLB to T317C-SERCA2a occurred only in the absence of Ca2+ and in the presence of nucleotide and was prevented by thapsigargin and by anti-PLB antibody, demonstrating for a fourth cross-linking pair that PLB interacts near M4 only when the Ca2+ pump is in the Ca2+-free, nucleotide-bound E2 conformation, but not in the E2 state inhibited by thapsigargin. L31I-PLB retained full functional and cross-linking activity, suggesting that a bulky hydrophobic residue at position 31 of PLB is essential for productive interaction with SERCA2a. A model for the three-dimensional structure of the interaction site is proposed.

2/7/2
DIALOG(R)File 5:Biosis Previews(R)

0015316227 BIOSIS NO.: 200510010727
Serine 16 phosphorylation induces an order-to-disorder transition in monomeric %%%phospholamban%%%
AUTHOR: Metcalfe Emily E; Traaseth Nathaniel J; Veglia Gianluigi (Reprint)
AUTHOR ADDRESS: Univ Minnesota, Dept Chem, 207 Pleasant St SE, Minneapolis, MN 55455 USA**USA
AUTHOR E-MAIL ADDRESS: veglia@chem.umn.edu
JOURNAL: Biochemistry 44 (11): p4386-4396 MAR 22 05 2005
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLB) is a 52 amino acid membrane-endogenous regulator of the sarco(endo)-plasmic calcium adenosinetriphosphatase (SERCA) in cardiac muscle. PLB's phosphorylation and dephosphorylation at S16 modulate its regulatory effect on SERCA by an undetermined mechanism. In this paper, we use multidimensional H-1/N-15 solution NMR methods to establish the structural and dynamics basis for PLB's control of SERCA upon S16 phosphorylation. For our studies, we use a monomeric, fully active mutant of PLB, where C36, C41, and C46 have been %%%mutated%%% to A36, F41, and A46, respectively. Our data show that phosphorylation disrupts the "L-shaped" structure of monomeric PLB, causing significant unwinding of both the cytoplasmic helix (domain la) and the short loop (residues 17-21) connecting this domain to the transmembrane helix (domains Ib and II). Concomitant with this conformational transition, we also find pronounced changes in both the pico- to nanosecond and the micro- to millisecond time scale dynamics. The H-1/N-15 heteronuclear NOE values for residues 1-25 are significantly lower than those of unphosphorylated PLB, with slightly lower NOE values in the transmembrane domain, reflecting less restricted motion throughout the whole protein. These data are supported by the faster spin-lattice relaxation rates (R-1) present in both the cytoplasmic and loop regions and by the enhanced spin-spin transverse relaxation rates (R-2) observed in the transmembrane domain. These results demonstrate that while S16 phosphorylation induces a localized structural transition, changes in PLB's backbone dynamics are propagated throughout the protein backbone. We propose that the regulatory mechanism of PLB phosphorylation involves an order-to-disorder transition, resulting in a decrease in the PLB inhibition of SERCA.

2/7/3
DIALOG(R)File 5:Biosis Previews(R)
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0015311682 BIOSIS NO.: 200510006182
De novo design of a pentameric coiled-coil: decoding the motif for tetramer versus pentamer formation in water-soluble %%%phospholamban%%%
AUTHOR: Slovic A M; Lear J D; DeGrado W F (Reprint)
AUTHOR ADDRESS: Univ Penn, Dept Biochem and Mol Biophys, Johnson Fdn, Sch Med, 1010 Stellar Chance Bldg 420 Curie Blvd, Philadelphia, PA 19104 USA **USA
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JOURNAL: Journal of Peptide Research 65 (3): p312-321 MAR 05 2005
ISSN: 1397-002X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Water-soluble %%%phospholamban%%% (WSPLB) is a designed, water-soluble analogue of the pentameric membrane protein %%%phospholamban%%% (PLB), which contains the same core and interhelical residues as PLB, with only the solvent-exposed positions %%%mutated%%%. WSPLB contains the same secondary and quaternary structure as PLB. The hydrophobic cores of PLB and WSPLB contain Leu and Ile at the a- and d-positions of a heptad repeat (abcdefg) from residues 31-52, while residues 21-30 are rich in polar amino acids at these positions. While the full-length WSPLB forms pentamers in solution, truncated peptides lacking residues 21-30 are largely tetrameric. Thus, truncation of residues 1-20 promotes a switch from pentamer to tetramer formation. Here, the motifs for WSPLB pentamerization were elucidated by characterizing a series of peptides, which were progressively truncated in this polar 'switch' region. When fully present, the 'switch' region promotes pentamer formation in WSPLB, by destabilizing a more stable tetrameric species which exists in its absence. We find that the burial of hydrogen bonding residues from 21 to 30 drives WSPLB from a tetramer to a pentamer, with direct implications for coiled-coil design.

2/7/4
DIALOG(R)File 5:Biosis Previews(R)
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0015306654 BIOSIS NO.: 200510001154
The effects of %%%mutation%%% on the regulatory properties of %%%phospholamban%%% in co-reconstituted membranes
AUTHOR: Trieber Catharine A; Douglas Jennifer L; Afara Michael; Young Howard S (Reprint)
AUTHOR ADDRESS: Univ Alberta, Dept Biochem, Edmonton, AB T6G 2H7, Canada** Canada
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JOURNAL: Biochemistry 44 (9): p3289-3297 MAR 8 05 2005
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Reconstitution into proteoliposomes is a powerful method for studying calcium transport in a chemically pure membrane environment. By use of this approach, we have studied the regulation of Ca2+ ATPase by %%%phospholamban%%% (PLB) as a function of calcium concentration and PLB %%%mutation%%%. Coreconstitution of PLB and Ca2+-ATPase revealed the expected effects of PLB on the apparent calcium affinity of Ca2+-ATPase (K-ca) and unexpected effects of PLB on maximal activity (V-max). Wild-type PLB, six loss-of-function mutants (L7A, R9E, 112A, N34A, 138A, L42A), and three gain-of-function mutants (N27A, L37A, and 140A) were evaluated for their effects on K-ca and V-max. With the loss-of-function mutants, their ability to shift K-Ca correlated with their ability to increase V-max,. A total loss-of-function mutant, N34A, had no effect on KCa of the calcium pump and produced only a marginal increase in V(ma)x

near-wild-type mutant, I12A, significantly altered both K-Ca and V-max of the calcium pump. With the gain-of-function mutants, their ability to shift KC, did not correlate with their ability to increase V-max The "super-shifting" mutants N27A, L37A, and 140A produced a large shift in KCa of the calcium pump; however, L37A decreased V-max while N27A and 140A increased V a,. For wild-type PLB, phosphorylation completely reversed the effect on K-Ca but had no effect on V-max,. We conclude that PLB increases V-max of Ca2+-ATPase, and that the magnitude of this effect is sensitive to %%%mutation%%%. The %%%mutation%%% sensitivity of PLB Asn(34) and Leu(37) identifies a region of the protein that is responsible for this regulatory property.

2/7/5
DIALOG(R)File 5:Biosis Previews(R)

0015304081 BIOSIS NO.: 200500207883
Evaluation of the %%%phospholamban%%% gene in purebred large-breed dogs with dilated cardiomyopathy
AUTHOR: Stabej Polona (Reprint); Leegwater Peter A; Stokhof Arnold A; Domanjko-Petric Aleksandra; van Oost Bernard A
AUTHOR ADDRESS: Dept Clin Sci, Univ Utrecht, NL-3584 CM, Utrecht, Netherlands**Netherlands
JOURNAL: American Journal of Veterinary Research 66 (3): p432-436 March 2005 2005
MEDIUM: print
ISSN: 0002-9645 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Objective-To evaluate the role of the %%%phospholamban%%% gene in purebred large-breed dogs with dilated cardiomyopathy (DCM). Animals-6 dogs with DCM, including 2 Doberman Pinschers, 2 Newfoundlands, and 2 Great Danes. Procedure-All dogs had clinical signs of congestive heart failure, and a diagnosis of DCM was made on the basis of echocardiographic findings. Blood samples were collected from each dog, and genomic DNA was isolated by a salt extraction method. Specific oligonucleotides were designed to amplify the promoter, exon 1, the 5'-part of exon 2 including the complete coding region, and part of intron I of the canine %%%phospholamban%%% gene via polymerase chain reaction procedures. These regions were screened for %%%mutations%%% in DNA obtained from the 6 dogs with DCM. Results-No %%%mutations%%% were identified in the promoter, 5' untranslated region, part of intron 1, part of the 3' untranslated region, and the complete coding region of the %%%phospholamban%%% gene in dogs with DCM. Conclusions and Clinical Relevance-Results indicate that %%%mutations%%% in the %%%phospholamban%%% gene are not a frequent cause of DCM in Doberman Pinschers, Newfoundlands, and Great Danes.

2/7/6
DIALOG(R)File 5:Biosis Previews(R)

0015261656 BIOSIS NO.: 200500168392
Novel %%%mutations%%% in %%%phospholamban%%% cause HCM and DCM
AUTHOR: Fujino Noboru; Shimizu Masami; Mabuchi Hiroshi
JOURNAL: Journal of Molecular and Cellular Cardiology 37 (5): p1083 November 2004 2004
MEDIUM: print
CONFERENCE/MEETING: 21st Annual Meeting of the Japanese Section of the International Society for Heart Research (ISHR) Kofu City, Yamanashi, Japan November 23-25, 2004; 20041123
SPONSOR: International Society for Heart Research
ISSN: 0022-2828 _(ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/7
DIALOG(R)File 5:Biosis Previews(R)

0015130405 BIOSIS NO.: 200500037470
Association between %%%mutation%%% of %%%phospholamban%%% gene and dilated cardiomyopathy
AUTHOR: Zhao Chun-xia (Reprint); Cui Ying-Hua; Zhu Zhi-Hui; Wang Dao-Wen
AUTHOR ADDRESS: Tongji Med CollTongji HospDept Internal Med, Huazhong Univ Sci and Technol, Wuhan, 430030, China**China
AUTHOR E-MAIL ADDRESS: zhaocx2001@yahoo.com.cn; dwwang@tjh.tjmu.edu.cn
JOURNAL: Yichuan 26 (5): p599-602 September 2004 2004
MEDIUM: print
ISSN: 0253-9772 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Chinese

ABSTRACT: %%%Phospholamban%%% (PLB) is a prominent regulator of myocardial contractility and a reversible inhibitor of the cardiac sarcoplasmic reticulum Ca2+ ATPase activity. In normal cardiac muscles, %%%phospholamban%%% can be phosphorylated at distinct sites by various protein kinases and release its inhibition to sarcoplasmic reticulum Ca2+ ATPase. The studies of pedigrees have shown dilated cardiomyopathy (DCM) is related with %%%mutation%%% of PLB gene. The aim of present study is to investigate the relationship between %%%mutation%%% of PLB gene and DCM. Sixty patients with idiotic DCM were enrolled in present study. The clinical data were collected, including clinical symptoms, ECG and echocardiography. Peripheral blood samples of all these subjects were collected to extract genome DNA. The fragments of PLB gene were amplified by PCR and PCR fragment sequencing was performed to study weather %%%mutation%%% of %%%phospholamban%%% gene exists. %%%Phospholamban%%% gene did not show any %%%mutation%%% in these patients. Most Chinese DCM patients may not be related with %%%mutation%%% of PLB gene.

2/7/8
DIALOG(R)File 5:Biosis Previews(R)

0015027958 BIOSIS NO.: 200400398747
Calcium cycling proteins in heart failure, cardiomyopathy and arrhythmias
AUTHOR: Minamisawa Susumu (Reprint); Sato Yoji; Cho Myeong-Chan
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JOURNAL: Experimental & Molecular Medicine 36 (3): p193-203 June 30, 2004 2004
MEDIUM: print
ISSN: 1226-3613 _(ISSN print)
DOCUMENT TYPE: Article; Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A growing body of evidence, including studies using genetically engineered mouse models, has shown that Ca2+ cycling and Ca2+-dependent signaling pathways play a pivotal role in cardiac hypertrophy and heart failure. In addition, recent studies identified that %%%mutations%%% of the genes encoding sarcoplasmic reticulum (SR) proteins cause human cardiomyopathies and lethal ventricular arrhythmias. The regulation of Ca2+ homeostasis via the SIR proteins may have potential therapeutic value for heart diseases such as cardiomyopathy, heart failure and arrhythmias.

2/7/9
DIALOG(R)File 5:Biosis Previews(R)

0014994822 BIOSIS NO.: 200400365611

Rescue of a mouse model of human dilated cardiomyopathy due to a missense %%%mutation%%% in %%%phospholamban%%%
AUTHOR: Schmitt J P; Hein L; Seidman J G; Seidman C E
JOURNAL: Naunyn-Schmiedeberg's Archives of Pharmacology 369 (Suppl. 1): p R88 March 2004 2004
MEDIUM: print
CONFERENCE/MEETING: 45th Spring Meeting of the Deutsche Gesellschaft fuer Experimentelle und Klinische Pharmakologie und Toxikologie Mainz, Germany March 09-11, 2004; 20040309
SPONSOR: Deutsche Gesellschaft fuer Experimentelle und Klinische Pharmakologie und Toxikologie
ISSN: 0028-1298 _(ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/10
DIALOG(R)File 5:Biosis Previews(R)

0014801385 BIOSIS NO.: 200400172142
Chronic %%%phospholamban%%% inhibition prevents progressive cardiac dysfunction and pathological remodeling after infarction in rats.
AUTHOR: Iwanaga Yoshitaka; Hoshijima Masahiko; Gu Yusu; Iwatate Mitsuo; Dieterle Thomas; Ikeda Yasuhiro; Date Moto-o; Chrast Jacqueline; Matsuzaki Masunori; Peterson Kirk L; Chien Kenneth R; Ross John (Reprint)
AUTHOR ADDRESS: Department of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA, 92093-0613, USA**USA
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JOURNAL: Journal of Clinical Investigation 113 (5): p727-736 March 2004 2004
MEDIUM: print
ISSN: 0021-9738
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Ablation or inhibition of %%%phospholamban%%% (PLN) has favorable effects in several genetic murine dilated cardiomyopathies, and we showed previously that a pseudophosphorylated form of PLN mutant (S16EPLN) successfully prevented progressive heart failure in cardiomyopathic hamsters. In this study, the effects of PLN inhibition were examined in rats with heart failure after myocardial infarction (MI), a model of acquired disease. S16EPLN was delivered into failing hearts 5 weeks after MI by transcoronary gene transfer using a recombinant adeno-associated virus (rAAV) vector. In treated (MI-S16EPLN, n=16) and control (MI-saline, n=18) groups, infarct sizes were closely matched and the left ventricle was similarly depressed and dilated before gene transfer. At 2 and 6 months after gene transfer, MI-S16EPLN rats showed an increase in left ventricular (LV) ejection fraction and a much smaller rise in LV end-diastolic volume, compared with progressive deterioration of LV size and function in MI-saline rats. Hemodynamic measurements at 6 months showed lower LV end-diastolic pressures, with enhanced LV function (contractility and relaxation), lowered LV mass and myocyte size, and less fibrosis in MI-S16EPLN rats. Thus, PLN inhibition by in vivo rAAV gene transfer is an effective strategy for the chronic treatment of an acquired form of established heart failure.

2/7/11
DIALOG(R)File 5:Biosis Previews(R)

0014785269 BIOSIS NO.: 200400151930
NMR studies of the interaction between %%%phospholamban%%% and the C subunit of protein kinase A.
AUTHOR: Mascioni Alessandro (Reprint); Veglia Gianluigi (Reprint)
AUTHOR ADDRESS: Chemistry, University of Minnesota, Minneapolis, MN, USA** USA
JOURNAL: Biophysical Journal 86 (1): p492a January 2004 2004

MEDIUM: print
CONFERENCE/MEETING: 48th Annual Meeting of the Biophysical Society
Baltimore, MD, USA February 14-18, 2004; 20040214
SPONSOR: Biophysical Society
ISSN: 0006-3495 _(ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLB) is a 52 residues membrane protein responsible for the regulation of the activity of the Ca-ATPase in the sarcoendoplasmic membranes in the cardiac muscle. PLB is further regulated by phosphorylation at S16 and T17 by the cAMP dependent protein kinase A (PKA). It has been recently reported that a malignant %%%mutation%%% of %%%phospholamban%%% with replacement of R9 with a cysteine (R9C-PLB), is correlated with hereditary dilated cardiomyopathy. R9C-PLB is unable to inhibit Ca-ATPase, and when co-transfected with wild-type PLB, it irreversibly bind PKA increasing the amount of unphosphorylated wild-type PLB. Therefore, it has been proposed that R9C-PLB is an inhibitor of PKA, hampering the phosphorylation of wt-PLB and impairing the Ca2+ transport across the membrane. In the present work we present preliminary solution and solid-state NMR data on the binding of wt-PLB and R9C-PLB with C subunit of PKA.

2/7/12
DIALOG(R)File 5:Biosis Previews(R)

0014692437 BIOSIS NO.: 200400063194
%%%Mutations%%% in the Neuregulin-1/ErbB2 signaling system cause dilated cardiomyopathy.
AUTHOR: Ozcelik Cemil (Reprint); Li Li (Reprint); Pilz Bernhard; Osterziel Karl Josef; Garratt Alistair N (Reprint); Birchmeier Carmen (Reprint)
AUTHOR ADDRESS: Max-Delbrueck-Cntr, Berlin, Germany**Germany
JOURNAL: Circulation 108 (17 Supplement): pIV-116 October 28, 2003 2003
MEDIUM: print
CONFERENCE/MEETING: American Heart Association Scientific Sessions 2003
Orlando, FL, USA November 09-12, 2003; 20031109
SPONSOR: American Heart Association
ISSN: 0009-7322 _(ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/13
DIALOG(R)File 5:Biosis Previews(R)

0014528386 BIOSIS NO.: 200300486043
NMR solution structure and topological orientation of monomeric %%%phospholamban%%% in dodecylphosphocholine micelles.
AUTHOR: Zamoon Jamillah; Mascioni Alessandro; Thomas David D; Veglia Gianluigi (Reprint)
AUTHOR ADDRESS: Dept. of Chemistry, University of Minnesota, 207 Pleasant St. S.E., Minneapolis, MN, 55455, USA**USA
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JOURNAL: Biophysical Journal 85 (4): p2589-2598 October 2003 2003
MEDIUM: print
ISSN: 0006-3495 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% is an integral membrane protein that regulates the contractility of cardiac muscle by maintaining cardiomyocyte calcium homeostasis. Abnormalities in association of protein kinase A with PLB have recently been linked to human heart failure, where a single %%%mutation%%% is responsible for dilated cardiomyopathy. To date, a high-resolution structure of

%%%phospholamban%%% in a lipid environment has been elusive. Here, we describe the first structure of recombinant, monomeric, biologically active %%%phospholamban%%% in lipid-mimicking dodecylphosphocholine micelles as determined by multidimensional NMR experiments. The overall structure of %%%phospholamban%%% is "L-shaped" with the hydrophobic domain approximately perpendicular to the cytoplasmic portion. This is in agreement with our previously published solid-state NMR data. In addition, there are two striking discrepancies between our structure and those reported previously for synthetic %%%phospholamban%%% in organic solvents: a), in our structure, the orientation of the cytoplasmic helix is consistent with the amphipathic nature of these residues; and b), within the hydrophobic helix, residues are positioned on two discrete faces of the helix as consistent with their functional roles ascribed by mutagenesis. This topology renders the two phosphorylation sites, Ser-16 and Thr-17, more accessible to kinases.

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DIALOG(R)File 5:Biosis Previews(R)

0014502033 BIOSIS NO.: 200300470752
Role of dual-site %%%phospholamban%%% phosphorylation in the stunned heart: Insights from %%%phospholamban%%% site-specific mutants.
AUTHOR: Said M; Vittone L; Mundina-Weilenmann C; Ferrero P; Kranias E G; Mattiazzi A (Reprint)
AUTHOR ADDRESS: Centro de Investigaciones Cardiovasculares, Facultad de Ciencias Medicas, 60 y 120, 1900, La Plata, Argentina**Argentina
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JOURNAL: American Journal of Physiology 285 (3 Part 2): pH1198-H1205 September 2003 2003
MEDIUM: print
ISSN: 0002-9513 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Phosphorylation of %%%phospholamban%%% (PLB) at Ser16 (protein kinase A site) and at Thr17 (Ca2+/calmodulin kinase II (CaMKII) site) increases sarcoplasmic reticulum Ca2+ uptake and myocardial contractility and relaxation. In perfused rat hearts submitted to ischemia-reperfusion, we previously showed an ischemia-induced Ser16 phosphorylation that was dependent on beta-adrenergic stimulation and an ischemia and reperfusion-induced Thr17 phosphorylation that was dependent on Ca2+ influx. To elucidate the relationship between these two PLB phosphorylation sites and postischemic mechanical recovery, rat hearts were submitted to ischemia-reperfusion in the absence and presence of the CaMKII inhibitor KN-93 (1 muM) or the beta-adrenergic blocker dl-propranolol (1 muM). KN-93 diminished the reperfusion-induced Thr17 phosphorylation and depressed the recovery of contraction and relaxation after ischemia. dl-Propranolol decreased the ischemia-induced Ser16 phosphorylation but failed to modify the contractile recovery. To obtain further insights into the functional role of the two PLB phosphorylation sites in postischemic mechanical recovery, transgenic mice expressing wild-type PLB (PLB-WT) or PLB mutants in which either Thr17 or Ser16 were replaced by Ala (PLB-T17A and PLB-S16A, respectively) into the PLB-null background were used. Both PLB mutants showed a lower contractile recovery than PLB-WT. However, this recovery was significantly impaired all along reperfusion in PLB-T17A, whereas it was depressed only at the beginning of reperfusion in PLB-S16A. Moreover, the recovery of relaxation was delayed in PLB-T17A, whereas it did not change in PLB-S16A, compared with PLB-WT. These findings indicate that, although both PLB phosphorylation sites are involved in the mechanical recovery after ischemia, Thr17 appears to play a major role.

2/7/15
DIALOG(R)File 5:Biosis Previews(R)

0014479536 BIOSIS NO.: 200300436570

Comparison of the backbone dynamics of %%%phospholamban%%% and Ser16-phosphorylated %%%phospholamban%%%.
AUTHOR: Metcalfe Emily E (Reprint); Zamoon Jamillah (Reprint); Pierson Debbie L (Reprint); Cornea Razvan L (Reprint); Thomas David D (Reprint); Veglia Gianluigi (Reprint)
AUTHOR ADDRESS: University of Minnesota, 207 Pleasant St SE, 139 Smith Hall, Minneapolis, MN, 55455, USA**USA
JOURNAL: Biophysical Journal 84 (2 Part 2): p275a February 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 47th Annual Meeting of the Biophysical Society San Antonio, TX, USA March 01-05, 2003; 20030301
SPONSOR: Biophysical Society
ISSN: 0006-3495 _(ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/16
DIALOG(R)File 5:Biosis Previews(R)

0014340858 BIOSIS NO.: 200300298677
Sarcolipin regulates sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) by binding to transmembrane helices alone or in association with %%%phospholamban%%%.
AUTHOR: Asahi Michio; Sugita Yuji; Kurzydlowski Kazimierz; de Leon Stella; Tada Michihiko; Toyoshima Chikashi; MacLennan David H (Reprint)
AUTHOR ADDRESS: Banting and Best Department of Medical Research, University of Toronto, Charles H. Best Institute, 112 College Street, Toronto, ON, M5G 1L6, Canada**Canada
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JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 100 (9): p5040-5045 April 29, 2003 2003
MEDIUM: print
ISSN: 0027-8424 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLN), a regulator of sarco(endo)plasmic reticulum Ca2+-ATPases (SERCAs), interacts with both the cytosolic N domain and transmembrane helices M2, M4, M6, and M9 of SERCA. Amino acids in the transmembrane domain of PLN that are predicted to interact with SERCA1a are conserved in sarcolipin (SLN), a functional PLN homologue. Accordingly, the effects of critical %%%mutations%%% in SERCA1a, PLN, and NF-SLN (SLN tagged N-terminally with a FLAG epitope) on NF-SLN/SERCA1a and PLN/NF-SLN/SERCA1a interactions were compared. Critical %%%mutations%%% in SERCA1a and NF-SLN diminished functional interactions between SERCA1a and NF-SLN, indicating that NF-SLN and PLN interact with some of the same amino acids in SERCA1a. %%%Mutations%%% in PLN or NF-SLN affected the amount of SERCA1a that was coimmunoprecipitated in each complex with antibodies against either PLN or SLN, but not the pattern of coimmunoprecipitation. PLN %%%mutations%%% had more dramatic effects on SERCA1a coimmunoprecipitation than SLN %%%mutations%%%, suggesting that PLN dominates in the primary interaction with SERCA1a. Coimmunoprecipitation also confirmed that PLN and NF-SLN form a heterodimer that interacts with SERCA1a in a regulatory fashion to form a very stable PLN/NF-SLN/SERCA1a complex. Modeling showed that the SLN/SERCA1a complex closely resembles the PLN/SERCA1a complex, but with the luminal end of SLN extending to the loop connecting M1 and M2, where Tyr-29 and Tyr-31 interact with aromatic residues in SERCA1a. Modeling of the PLN/SLN/SERCA1a complex predicts that the regulator binding cavity in the E2 conformation of SERCA1a can accommodate both SLN and PLN helices, but not two PLN helices.

2/7/17
DIALOG(R)File 5:Biosis Previews(R)

0014340651 BIOSIS NO.: 200300298470
%%%Mutation%%% of the %%%phospholamban%%% promoter associated with hypertrophic cardiomyopathy.
AUTHOR: Minamisawa Susumu; Sato Yoji; Tatsuguchi Yuriko; Fujino Tomofumi; Imamura Shin-ichiro; Uetsuka Yoshio; Nakazawa Makoto; Matsuoka Rumiko (Reprint)
AUTHOR ADDRESS: Department of Pediatric Cardiology, Heart Institute of Japan, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo, 162-8666, Japan**Japan
AUTHOR E-MAIL ADDRESS: sminamis@med.yokohama-cu.ac.jp; rumiko@imcir.twmu.ac.jp
JOURNAL: Biochemical and Biophysical Research Communications 304 (1): p1-4 April 25, 2003 2003
MEDIUM: print
ISSN: 0006-291X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% is an endogenous inhibitor of sarcoplasmic reticulum calcium ATPase and plays a prime role in cardiac contractility and relaxation. %%%Phospholamban%%% may be a candidate gene responsible for cardiomyopathy. We investigated genome sequence of %%%phospholamban%%% in patients with cardiomyopathy. PCR-based direct sequence was performed for the promoter region and the whole coding region of %%%phospholamban%%% in 87 hypertrophic, 10 dilated, and 2 restricted cardiomyopathic patients. We found a heterozygous single nucleotide transition from A to G at -77-bp upstream of the transcription start site in the %%%phospholamban%%% promoter region of one patient with familial hypertrophic cardiomyopathy. This nucleotide change was not found in 296 control subjects. Using neonatal rat cardiomyocytes, the %%%mutation%%%, -77A fwdarw G, increased the %%%phospholamban%%% promoter activity. No nucleotide change in the %%%phospholamban%%% coding region was found in 99 patients with cardiomyopathy. We suspect that the %%%mutation%%% plays an important role in the development of hypertrophic cardiomyopathy.

2/7/18
DIALOG(R)File 5:Biosis Previews(R)

0014298430 BIOSIS NO.: 200300257074
Human %%%phospholamban%%% null results in lethal dilated cardiomyopathy revealing a critical difference between mouse and human.
AUTHOR: Haghighi Kobra; Kolokathis Fotis; Pater Luke; Lynch Roy A; Asahi Michio; Gramolini Anthony O; Fan Guo-chang; Tsiapras Dimitris; Hahn Harvey S; Adamopoulos Stamatis; Liggett Stephen B; Dorn Gerald W; MacLennan David H; Kremastinos Dimitrios T; Kranias Evangelia G (Reprint)
AUTHOR ADDRESS: Department of Pharmacology and Cell Biophysics, College of Medicine, University of Cincinnati, 231 Albert Sabin Way, Cincinnati, OH, 45267-0575, USA**USA
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JOURNAL: Journal of Clinical Investigation 111 (6): p869-876 March 2003 2003
MEDIUM: print
ISSN: 0021-9738
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In human disease and experimental animal models, depressed Ca2+ handling in failing cardiomyocytes is widely attributed to impaired sarcoplasmic reticulum (SR) function. In mice, disruption of the PLN gene encoding %%%phospholamban%%% (PLN) or expression of dominant-negative PLN mutants enhances SR and cardiac function, but effects of PLN %%%mutations%%% in humans are unknown. Here, a T116G point %%%mutation%%%, substituting a termination codon for Leu-39 (L39stop), was identified in two families with hereditary heart failure. The heterozygous individuals exhibited hypertrophy without diminished contractile performance. Strikingly, both individuals homozygous for L39stop

developed dilated cardiomyopathy and heart failure, requiring cardiac transplantation at ages 16 and 27. An over 50% reduction in PLN mRNA and no detectable PLN protein were noted in one explanted heart. The expression of recombinant PLN-L39stop in human embryonic kidney (HEK) 293 cells and adult rat cardiomyocytes showed no PLN inhibition of SR Ca2+-ATPase and the virtual absence of stable PLN expression; where PLN was expressed, it was misrouted to the cytosol or plasma membrane. These findings describe a naturally-occurring loss-of-function human PLN %%%mutation%%% (PLN null). In contrast to reported benefits of PLN ablation in mouse heart failure, humans lacking PLN develop lethal dilated cardiomyopathy.

2/7/19
DIALOG(R)File 5:Biosis Previews(R)

0014298428 BIOSIS NO.: 200300257072
Rescue of cardiomyocyte dysfunction by %%%phospholamban%%% ablation does not prevent ventricular failure in genetic hypertrophy.
AUTHOR: Song Qiujing; Schmidt Albrecht G; Hahn Harvey S; Carr Andrew N; Frank Beate; Pater Luke; Gerst Mike; Young Karen; Hoit Brian D; McConnell Bradley K; Haghighi Kobra; Seidman Christine E; Seidman Jonathan G; Dorn Gerald W; Kranias Evangelia G (Reprint)
AUTHOR ADDRESS: Department of Pharmacology and Cell Biophysics, University of Cincinnati Medical Center, 231 Albert Sabin Way, Cincinnati, OH, 45267-0575, USA**USA
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JOURNAL: Journal of Clinical Investigation 111 (6): p859-867 March 2003 2003
MEDIUM: print
ISSN: 0021-9738
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Cardiac hypertrophy, either compensated or decompensated, is associated with cardiomyocyte contractile dysfunction from depressed sarcoplasmic reticulum (SR) Ca2+ cycling. Normalization of Ca2+ cycling by ablation or inhibition of the SR inhibitor %%%phospholamban%%% (PLN) has prevented cardiac failure in experimental dilated cardiomyopathy and is a promising therapeutic approach for human heart failure. However, the potential benefits of restoring SR function on primary cardiac hypertrophy, a common antecedent of human heart failure, are unknown. We therefore tested the efficacy of PLN ablation to correct hypertrophy and contractile dysfunction in two well-characterized and highly relevant genetic mouse models of hypertrophy and cardiac failure, Galphaq overexpression and human familial hypertrophic cardiomyopathy mutant myosin binding protein C (MyBP-CMUT) expression. In both models, PLN ablation normalized the characteristically prolonged cardiomyocyte Ca2+ transients and enhanced unloaded fractional shortening with no change in SR Ca2+ pump content. However, there was no parallel improvement in in vivo cardiac function or hypertrophy in either model. Likewise, the activation of JNK and calcineurin associated with Galphaq overexpression was not affected. Thus, PLN ablation normalized contractility in isolated myocytes, but failed to rescue the cardiomyopathic phenotype elicited by activation of the Galphaq pathway or MyBP-C %%%mutations%%%.

2/7/20
DIALOG(R)File 5:Biosis Previews(R)

0014271628 BIOSIS NO.: 200300228428
Absence of %%%mutation%%% in 4 candidate genes in a large European population of dilated cardiomyopathy.
AUTHOR: Villard E (Reprint); Sylvius N (Reprint); Duboscq-Bidot L (Reprint); Bouchier C (Reprint); Sebillon P (Reprint); Charron P; Isnard R; Komajda M
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JOURNAL: European Heart Journal 23 (Abstract Supplement): p393
August-September 2002 2002
MEDIUM: print
CONFERENCE/MEETING: Congress of the European Society of Cardiology Berlin, Germany August 31-September 04, 2002; 20020831
SPONSOR: European Society of Cardiology
ISSN: 0195-668X _(ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/21
DIALOG(R)File 5:Biosis Previews(R)

0014195225 BIOSIS NO.: 200300153944
Dilated cardiomyopathy and heart failure caused by a %%%mutation%%% in %%%phospholamban%%%.
AUTHOR: Schmitt Joachim P; Kamisago Mitsuhiro; Asahi Michio; Li Guo Hua; Ahmad Ferhaan; Mende Ulrike; Kranias Evangelia G; MacLennan David H; Seidman J G; Seidman Christine E (Reprint)
AUTHOR ADDRESS: Department of Genetics, Harvard Medical School and Howard Hughes Medical Institute, 200 Longwood Avenue, Boston, MA, 02115, USA** USA
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JOURNAL: Science (Washington D C) 299 (5611): p1410-1413 28 February, 2003 2003
MEDIUM: print
ISSN: 0036-8075 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Molecular etiologies of heart failure, an emerging cardiovascular epidemic affecting 4.7 million Americans and costing 17.8 billion health-care dollars annually, remain poorly understood. Here we report that an inherited human dilated cardiomyopathy with refractory congestive heart failure is caused by a dominant Arg fwdarw Cys missense %%%mutation%%% at residue 9 (R9C) in %%%phospholamban%%% (PLN), a transmembrane phosphoprotein that inhibits the cardiac sarcoplasmic reticular Ca2+-adenosine triphosphatase (SERCA2a) pump. Transgenic PLNR9C mice recapitulated human heart failure with premature death. Cellular and biochemical studies revealed that, unlike wild-type PLN, PLNR9C did not directly inhibit SERCA2a. Rather, PLNR9C trapped protein kinase A (PKA), which blocked PKA-mediated phosphorylation of wild-type PLN and in turn delayed decay of calcium transients in myocytes. These results indicate that myocellular calcium dysregulation can initiate human heart failure-a finding that may lead to therapeutic opportunities.

2/7/22
DIALOG(R)File 5:Biosis Previews(R)

0014168509 BIOSIS NO.: 200300125619
Computational design of a water-soluble analog of %%%phospholamban%%%.
AUTHOR: Slovic Avram M; Summa Christopher M; Lear James D; DeGrado William F (Reprint)
AUTHOR ADDRESS: Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104-6059, USA**USA
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JOURNAL: Protein Science 12 (2): p337-348 February 2003 2003
MEDIUM: print
ISSN: 0961-8368
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Membrane proteins and water-soluble proteins share a similar core. This similarity suggests that it should be possible to

water-solubilize membrane proteins by %%%mutating%%% only their lipid-exposed residues. We have developed computational tools to design water-soluble variants of helical membrane proteins, using the pentameric %%%phospholamban%%% (PLB) as our test case. To water-solublize PLB, the membrane-exposed positions were changed to polar or charged amino acids, while the putative core was left unaltered. We generated water-soluble %%%phospholamban%%% (WSPLB), and compared its properties to its predecessor PLB. In aqueous solution, WSPLB mimics all of the reported properties of PLB including oligomerization state, helical structure, and stabilization upon phosphorylation. We also characterized the truncated mutant WSPLB (21-52) comprising only the former transmembrane segment of PLB. This peptide shows a decreased specificity for forming a pentameric oligomerization state.

2/7/23
DIALOG(R)File 5:Biosis Previews(R)

0014120225 BIOSIS NO.: 200300078944
%%%Phospholamban%%% missense %%%mutation%%% Arg9Cys causes dominant dilated cardiomyopathy in man and mouse.
AUTHOR: Schmitt Joachim P (Reprint); Kamisago Mitsuhiro; Asahi Michio; Li Guo Hua; Ahmad Ferhaan; MacLennan David H; Seidman Jonathan; Seidman Christine
AUTHOR ADDRESS: Dept of Genetics, Boston, MA, USA**USA
JOURNAL: Circulation 106 (19 Supplement): pII-7 November 5, 2002 2002
MEDIUM: print
CONFERENCE/MEETING: Abstracts from Scientific Sessions Chicago, IL, USA November 17-20, 2002; 20021117
SPONSOR: American Heart Association
ISSN: 0009-7322 _(ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/24
DIALOG(R)File 5:Biosis Previews(R)

0013928395 BIOSIS NO.: 200200521906
Solid-state NMR and rigid body molecular dynamics to determine domain orientations of monomeric %%%phospholamban%%%
AUTHOR: Mascioni Alessandro; Karim Christine; Zamoon Jamillah; Thomas David D; Veglia Gianluigi (Reprint)
AUTHOR ADDRESS: Department of Chemistry, University of Minnesota, Minneapolis, MN, 55455, USA**USA
JOURNAL: Journal of the American Chemical Society 124 (32): p9392-9393 August 14, 2002 2002
MEDIUM: print
ISSN: 0002-7863
DOCUMENT TYPE: Article
RECORD TYPE: Citation
LANGUAGE: English

2/7/25
DIALOG(R)File 5:Biosis Previews(R)

0013885404 BIOSIS NO.: 200200478915
Close proximity between residue 30 of %%%phospholamban%%% and cysteine 318 of the cardiac Ca2+ pump revealed by intermolecular thiol cross-linking
AUTHOR: Jones Larry R (Reprint); Cornea Razvan L; Chen Zhenhui
AUTHOR ADDRESS: Krannert Institute of Cardiology, 1800 N. Capitol Ave., Indianapolis, IN, 46202, USA**USA
JOURNAL: Journal of Biological Chemistry 277 (31): p28319-28329 August 2, 2002 2002
MEDIUM: print
ISSN: 0021-9258

DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLB) is a 52-amino acid inhibitor of the Ca2+-ATPase in cardiac sarcoplasmic reticulum (SERCA2a), which acts by decreasing the apparent affinity of the enzyme for Ca2+. To localize binding sites of SERCA2a for PLB, we performed Cys-scanning mutagenesis of PLB, co-expressed the PLB mutants with SERCA2a in insect cell microsomes, and tested for cross-linking of the %%%mutated%%% PLB molecules to SERCA2a using 1,6-bismaleimidohexane, a 10-ANG-long, homobifunctional thiol cross-linking agent. Of several mutants tested, only PLB with a Cys replacement at position 30 (N30C-PLB) cross-linked to SERCA2a. Cross-linking occurred specifically and with high efficiency. The process was abolished by micromolar Ca2+ or by an anti-PLB monoclonal antibody and was inhibited 50% by phosphorylation of PLB by cAMP-dependent protein kinase. The SERCA2a inhibitors thapsigargin and cyclopiazonic acid also completely prevented cross-linking. The two essential requirements for cross-linking of N30C-PLB to SERCA2a were a Ca2+-free enzyme and, unexpectedly, a micromolar concentration of ATP or ADP, demonstrating that N30C-PLB cross-links preferentially to the nucleotide-bound, E2 state of SERCA2a. Sequencing of a purified proteolytic fragment in combination with SERCA2a mutagenesis identified Cys318 of SERCA2a as the sole amino acid cross-linked to N30C-PLB. The proximity of residue 30 of PLB to Cys318 of SERCA2a suggests that PLB may interfere with Ca2+ activation of SERCA2a by a protein interaction occurring near transmembrane helix M4.

2/7/26
DIALOG(R)File 5:Biosis Previews(R)

0013799780 BIOSIS NO.: 200200393291
Structure of the 1-36 N-terminal fragment of human %%%phospholamban%%% phosphorylated at Ser-16 and Thr-17
AUTHOR: Pollesello Piero (Reprint); Annila Arto
AUTHOR ADDRESS: Cardiovascular Research, Orion Pharma, FIN-02101, P.O. Box 65, Espoo, Finland**Finland
JOURNAL: Biophysical Journal 83 (1): p484-490 July, 2002 2002
MEDIUM: print
ISSN: 0006-3495
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The structure of a 36-amino-acid-long N-terminal fragment of human %%%phospholamban%%% phosphorylated at Ser-16 and Thr-17 and Cys-36fwdarwSer %%%mutated%%% was determined from nuclear magnetic resonance data in aqueous solution containing 30% trifluoroethanol. The peptide assumes a conformation characterized by two alpha-helices connected by an irregular strand, which comprises the amino acids from Arg-13 to Pro-21. The proline is in a trans conformation. The two phosphate groups on Ser-16 and Thr-17 are shown to interact preferably with the side chains of Arg-14 and Arg-13, respectively. The helix comprising amino acids 22 to 35 is well determined (the rmsd for the backbone atoms, calculated for a family of 24 nuclear magnetic resonance structures is 0.69+-0.28 ANG). The structures of phosphorylated and unphosphorylated %%%phospholamban%%% are compared, and the effect of the two phosphate groups on the relative spatial position of the two helices is examined. The packing parameters OMEGA (interhelical angle) and d (minimal interhelical distance) are calculated: in the case of the phosphorylated %%%phospholamban%%%, OMEGA=100+-35degree and d=7.9+-4.6 ANG, whereas for the unphosphorylated peptide the values are OMEGA=80+-20degree and d=7.0+-4.0 ANG. We conclude that 1) the phosphorylation does not affect the structure of the C terminus between residues 21 and 36 and 2) the phosphorylated %%%phospholamban%%% has more loose helical packing than the nonphosphorylated.

2/7/27

DIALOG(R)File 5:Biosis Previews(R)

0013728573 BIOSIS NO.: 200200322084
%%%Phospholamban%%% increases the VMAX of the Ca-ATPase in a reconstitution system at high PLB/Ca-ATPase ratios
AUTHOR: Reddy Laxma G (Reprint); Winters Deborah L (Reprint); McKenna Edward; Thomas David D (Reprint)
AUTHOR ADDRESS: University of Minnesota, 321 Church Street, SE, 6-155 Jackson Hall, Minneapolis, MN, 55455, USA**USA
JOURNAL: Biophysical Journal 82 (1 Part 2): p263a January, 2002 2002
MEDIUM: print
CONFERENCE/MEETING: 46th Annual Meeting of the Biophysical Society San Francisco, California, USA February 23-27, 2002; 20020223
ISSN: 0006-3495
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/28
DIALOG(R)File 5:Biosis Previews(R)

0013670154 BIOSIS NO.: 200200263665
Protein kinase A phosphorylation of the ryanodine receptor does not affect calcium sparks in permeabilized mouse ventricular myocytes
AUTHOR: Li Yanxia (Reprint); Bers Donald M (Reprint)
AUTHOR ADDRESS: Loyola Univ Med Ctr, Maywood, IL, USA**USA
JOURNAL: Circulation 104 (17 Supplement): pII.131 October 23, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: Scientific Sessions 2001 of the American Heart Association Anaheim, California, USA November 11-14, 2001; 20011111
SPONSOR: American Heart Association
ISSN: 0009-7322
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/29
DIALOG(R)File 5:Biosis Previews(R)

0013669926 BIOSIS NO.: 200200263437
%%%Phospholamban%%% ablation rescues intrinsic contractility but does not prevent remodeling in homozygous myosin-binding protein-C mutant mice
AUTHOR: Schmidt Albrecht G (Reprint); Carr Andrew N (Reprint); Frank Beate (Reprint); Gerst Michael J (Reprint); Pater Luke (Reprint); Hahn Harvey (Reprint); Hoit Brian D; McConnell Bradley K; Seidman J G G; Kranias Evangelia G
AUTHOR ADDRESS: Univ of Cincinnati, Cincinnati, OH, USA**USA
JOURNAL: Circulation 104 (17 Supplement): pII.83 October 23, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: Scientific Sessions 2001 of the American Heart Association Anaheim, California, USA November 11-14, 2001; 20011111
SPONSOR: American Heart Association
ISSN: 0009-7322
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/30
DIALOG(R)File 5:Biosis Previews(R)

0013669768 BIOSIS NO.: 200200263279
%%%Phospholamban%%% domain IB forms an interaction site with the loop between transmembrane helices M6 and M7 of sarco(endo)plasmic reticulum Ca2+ ATPase

AUTHOR: Asahi Michio (Reprint); Green N Michael; Kurzydlowski Kazimierz; Tada Michihiko; MacLennan David H
AUTHOR ADDRESS: Best Inst, Univ of Toronto, Toronto, ON, Canada**Canada
JOURNAL: Circulation 104 (17 Supplement): pII.50 October 23, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: Scientific Sessions 2001 of the American Heart Association Anaheim, California, USA November 11-14, 2001; 20011111
SPONSOR: American Heart Association
ISSN: 0009-7322
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/31
DIALOG(R)File 5:Biosis Previews(R)

0013668686 BIOSIS NO.: 200200262197
Phosphorylation of troponin I controls cardiac twitch dynamics: Evidence from phosphorylation site mutants expressed on a troponin I-null background in mice
AUTHOR: Pi YeQing; Kemnitz Kara R; Zhang Dahua; Kranias Evangelia G; Walker Jeffery W (Reprint)
AUTHOR ADDRESS: Dept of Physiology, 1300 University Ave, Madison, WI, 53706, USA**USA
JOURNAL: Circulation Research 90 (6): p649-656 April 5, 2002 2002
MEDIUM: print
ISSN: 0009-7330
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The cardiac myofilament protein troponin I (cTnI) is phosphorylated by protein kinase C (PKC), a family of serine/threonine kinases activated within heart muscle by a variety of agonists. cTnI is also a substrate for cAMP-dependent protein kinase (PKA) activated during beta-adrenergic signaling. To investigate the role of cTnI phosphorylation in contractile regulation by these pathways, we generated transgenic mice harboring a %%%mutated%%% cTnI protein lacking phosphorylation sites for PKC (serine43/45 and threonine144 %%%mutated%%% to alanine) and for PKA (serine23/24 %%%mutated%%% to alanine). Transgenic mice were interbred with cTnI-knockout mice to ensure the absence of endogenous phosphorylatable cTnI. Here, we report that regulation of myocyte twitch kinetics by beta-stimulation and by endothelin-1 was altered in myocytes containing mutant cTnI. In wild-type myocytes, the beta-agonist isoproterenol decreased twitch duration and relaxation time constant (tau) by 37% to 44%. These lusitropic effects of isoproterenol were reduced by about half in nonphosphorylatable cTnI mutant myocytes and were absent in cTnI mutants also lacking %%%phospholamban%%% (generated by crossing cTnI mutants with %%%phospholamban%%%-knockout mice). These observations are consistent with important roles for both cTnI and %%%phospholamban%%% phosphorylation in accelerating relaxation after beta-adrenergic stimulation. In contrast, endothelin-1 increased twitch duration by 32% and increased tau by 58%. These endothelin-1 effects were substantially blunted in nonphosphorylatable cTnI myocytes, indicating that PKC phosphorylation of cTnI slows cardiac relaxation and increases twitch duration. We propose that beta-agonists and endothelin-1 regulate cardiac twitch dynamics in opposite directions in part through phosphorylation of the myofilament protein cTnI on distinct sites.

2/7/32
DIALOG(R)File 5:Biosis Previews(R)

0013353700 BIOSIS NO.: 200100525539
Mapping the energy surface of transmembrane helix-helix interactions
AUTHOR: Torres Jaume; Kukol Andreas; Arkin Isaiah T (Reprint)
AUTHOR ADDRESS: Dept. of Biological Chemistry, Institute of Life Sciences,

The Hebrew University, Givat-Ram, Jerusalem, 91904, Israel**Israel
JOURNAL: Biophysical Journal 81 (5): p2681-2692 November, 2001 2001
MEDIUM: print
ISSN: 0006-3495
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Transmembrane helices are no longer believed to be just hydrophobic segments that exist solely to anchor proteins to a lipid bilayer, but rather they appear to have the capacity to specify function and structure. Specific interactions take place between hydrophobic segments within the lipid bilayer whereby subtle %%%mutations%%% that normally would be considered innocuous can result in dramatic structural differences. That such specificity takes place within the lipid bilayer implies that it may be possible to identify the most favorable interaction surface of transmembrane alpha-helices based on computational methods alone, as shown in this study. Herein, an attempt is made to map the energy surface of several transmembrane helix-helix interactions for several homo-oligomerizing proteins, where experimental data regarding their structure exist (glycophorin A, %%%phospholamban%%%, Influenza virus A M2, Influenza virus C CM2, and HIV vpu). It is shown that due to symmetry constraints in homo-oligomers the computational problem can be simplified. The results obtained are mostly consistent with known structural data and may additionally provide a view of possible alternate and intermediate configurations.

2/7/33
DIALOG(R)File 5:Biosis Previews(R)

0013147073 BIOSIS NO.: 200100318912
Conversion of %%%phospholamban%%% into a soluble pentameric helical bundle
AUTHOR: Li Huiming; Cocco Melanie J; Steitz Thomas A; Engelman Donald M (Reprint)
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JOURNAL: Biochemistry 40 (22): p6636-6645 June 5, 2001 2001
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LANGUAGE: English

ABSTRACT: Although membrane proteins and soluble proteins may achieve their final folded states through different pathways, it has been suggested that the packing inside a membrane protein could maintain a similar fold if the lipid-exposed surface were redesigned for solubility in an aqueous environment. To test this idea, the surface of the transmembrane domain of %%%phospholamban%%% (PLB), a protein that forms a stable helical homopentamer within the sarcoplasmic reticulum membrane, has been redesigned by replacing its lipid-exposed hydrophobic residues with charged and polar residues. CD spectra indicate that the full-length soluble PLB is highly alpha-helical. Small-angle X-ray scattering and multiangle laser light scattering experiments reveal that this soluble variant of PLB associates as a pentamer, preserving the oligomeric state of the natural protein. %%%Mutations%%% that destabilize native PLB also disrupt the pentamer. However, NMR experiments suggest that the redesigned protein exhibits molten globule-like properties, possibly because the redesign of the surface of this membrane protein may have altered some native contacts at the core of the protein or possibly because the core is not rigidly packed in wild-type PLB. Nonetheless, our success in converting the membrane protein PLB into a specific soluble helical pentamer indicates that the interior of a membrane protein contains at least some of the determinants necessary to dictate folding in an aqueous environment. The design we successfully used was based on one of the two models in the literature; the alternative design did not give stable, soluble pentamers. This suggests that surface redesign can be employed in gaining insights into the structures of membrane proteins.

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DIALOG(R)File 5:Biosis Previews(R)

0013092593 BIOSIS NO.: 200100264432
Cardiac overexpression of a %%%phospholamban%%% "supershifter" is associated with depressed cardiovascular function in transgenic mice
AUTHOR: Kirchhefer Uwe (Reprint); Jones Larry R; Schmitz Wilhelm (Reprint); Neumann Joachim (Reprint)
AUTHOR ADDRESS: Institut fuer Pharmakologie und Toxikologie, Universitaet Muenster, Domagkstr. 12, Muenster, 48149, Germany**Germany
JOURNAL: FASEB Journal 15 (4): pA478 March 7, 2001 2001
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CONFERENCE/MEETING: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001; 20010331
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DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLB) is a transmembrane protein of the sarcoplasmic reticulum (SR) which modulates the activity of the SR Ca2+-ATPase and basal contractility of the heart. Recent studies revealed that monomeric forms of PLB (e.g., L37A) are potent inhibitors of the Ca2+ transport in expression systems and transgenic models. However, other groups produced a strong inhibition of the SR-Ca2+-ATPase activity by pentameric forms of PLB (e.g., N27A). To determine whether simultaneous %%%mutation%%% at the two sites would be additive in their physiological effects, we generated transgenic mice overexpressing the double mutant N27A/L37A of PLB under the control of the cardiac-specific alpha-MHC promoter. Western blotting indicated a 2.5-fold increase in the total protein expression level of both monomeric and pentameric PLB in transgenic hearts. The Ca2+-uptake protein Ca2+-ATPase was downregulated, whereas the protein expression of triadin 1, a protein of the junctional SR which is associated with the Ca2+ release process, was enhanced in transgenic hearts. The apparent Ca2+ affinity of the Ca2+ pump was decreased in transgenic hearts compared to wild-type hearts. Isolated left atrial preparations were used to investigate contractile properties. The basal force of contraction was decreased by 46.2% in transgenic hearts. Moreover, time parameters (time of 90% contraction and time of 90% relaxation) were prolonged in transgenic compared to wild-type hearts. Isoprenaline (10-7 M) enhanced force of contraction 4.8-fold in transgenic and 1.9-fold in wild-type hearts. We conclude that the overexpression of the double mutant N27A/L37A-PLB is associated with impaired contractility in transgenic mice.

2/7/35
DIALOG(R)File 5:Biosis Previews(R)

0013085783 BIOSIS NO.: 200100257622
Loss of function of alpha-B-crystallin enhances the pathogenesis of a desmin %%%mutation%%%
AUTHOR: Wang Xuejun (Reprint); Robbins Jeffrey
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JOURNAL: FASEB Journal 15 (5): pA1158 March 8, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001; 20010331
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LANGUAGE: English

ABSTRACT: Alpha-B-crystallin (CryAB), a small heat shock protein, is abundant in not only the lens but also non-lenticular tissues such as

cardiac and skeletal muscle. It can bind to desmin and actin and shows molecular chaperone function in vitro. Like %%%mutations%%% of desmin, a missense %%%mutation%%% (R120G) of CryAB has recently been linked to desmin-related myopathy (DRM). It is thus believed that CryAB protects desmin filaments from stress damage. Previously we created a mouse model of desmin-related cardiomyopathy (DRC) via transgenic (TG) expression of a 7-amino-acid deletion (R173-E179) desmin in the heart. The mice show abnormal aggregation of cardiac desmin and early concentric cardiac hypertrophy and dysfunction. However, the life span of these mice appears to be normal. Interestingly, CryAB mRNA and protein were significantly increased in this model. To define the possible role(s) of increased CryAB in the pathogenesis of the desmin %%%mutation%%%, we generated additional TG mouse lines that express either wild type (WT) or a dominant negative (R120G) murine CryAB specifically in the heart. A DRC was not detected in any of the multiple WT-CryAB TG lines, indicating that WT-CryAB is not a direct mediator in the desmin pathogenesis. When the desmin mutant mice were bred into the R120G-CryAB background, the resultant double TG mice developed a more severe DRC and died of congestive heart failure at approximately 1 month of age. The double TG mice showed more cardiac hypertrophy, higher atrial natriuretic factor (ANF) and beta-myosin heavy chain (MyHC), and lower alpha-MyHC, sarcoplasmic reticulum calcium ATPase-2a, and %%%phospholamban%%% mRNA levels than their single TG littermates. The desmin aggregates were significantly increased in the double TG heart.

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DIALOG(R)File 5:Biosis Previews(R)

0013036959 BIOSIS NO.: 200100208798
Sarco(endo)plasmic reticulum calcium pumps: Recent advances in our understanding of structure/function and biology (Review)
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JOURNAL: Molecular Membrane Biology 17 (4): p189-200 October-December, 2000 2000
MEDIUM: print
ISSN: 0968-7688
DOCUMENT TYPE: Article; Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: This review examines the structure and function of the sarco(endo)plasmic reticulum calcium pump (SERCA1a) in the light of the recent publication of the 2.6 ANG resolution structure of this protein, and looks at the increasing awareness of the key role played by SERCAs in calcium signalling. The roles played by the calcium pump isoforms, SERCA1a/b, SERCA2a/b and SERCA3a/b/c in cellular function are discussed, and the modulation of SERCA activity by %%%phospholamban%%%, sarcolipin and other modulatory influences is examined. The recent discoveries of human SERCA %%%mutations%%% leading to disease states is reviewed, and the insights into SERCA function using transgenic approaches are outlined.

2/7/37
DIALOG(R)File 5:Biosis Previews(R)

0012940430 BIOSIS NO.: 200100112269
Aberrant desmin aggregation disrupts desmin filament networks and leads to early concentric cardiac hypertrophy in transgenic mice
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JOURNAL: Circulation 102 (18 Supplement): pII.290 October 31, 2000 2000
MEDIUM: print
CONFERENCE/MEETING: Abstracts from American Heart Association Scientific

Sessions 2000 New Orleans, Louisiana, USA November 12-15, 2000; 20001112
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LANGUAGE: English

2/7/38
DIALOG(R)File 5:Biosis Previews(R)

0012899061 BIOSIS NO.: 200100070900
Disruption of a single copy of the SERCA2 gene results in altered Ca2+ homeostasis and cardiomyocyte function
AUTHOR: Ji Yong; Lalli M Jane; Babu Gopal J; Xu Yanfang; Kirkpatrick Darryl L; Liu Lynne H; Chiamvimonvat Nipavan; Walsh Richard A; Shull Gary E; Periasamy Muthu (Reprint)
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JOURNAL: Journal of Biological Chemistry 275 (48): p38073-38080 December 1, 2000 2000
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A mouse model carrying a null %%%mutation%%% in one copy of the sarcoplasmic reticulum (SR) Ca2+-ATPase isoform 2 (SERCA2) gene, in which SERCA2 protein levels are reduced by apprx35%, was used to investigate the effects of decreased SERCA2 level on intracellular Ca2+ homeostasis and contractile properties in isolated cardiomyocytes. When compared with wild-type controls, SR Ca2+ stores and Ca2+ release in myocytes of SERCA2 heterozygous mice were decreased by apprx40-60% and apprx30-40%, respectively, and the rate of myocyte shortening and relengthening were each decreased by apprx40%. However, the rate of Ca2+ transient decline (tau) was not altered significantly, suggesting that compensation was occurring in the removal of Ca2+ from the cytosol. %%%Phospholamban%%%, which inhibits SERCA2, was decreased by apprx40% in heterozygous hearts, and basal phosphorylation of Ser-16 and Thr-17, which relieves the inhibition, was increased apprx2- and 2.1-fold. These results indicate that reduced expression and increased phosphorylation of %%%phospholamban%%% provides compensation for decreased SERCA2 protein levels in heterozygous heart. Furthermore, both expression and current density of the sarcolemmal Na+-Ca2+ exchanger were up-regulated. These results demonstrate that a decrease in SERCA2 levels can directly modify intracellular Ca2+ homeostasis and myocyte contractility. However, the resulting deficit is partially compensated by alterations in %%%phospholamban%%%/SERCA2 interactions and by up-regulation of the Na+-Ca2+ exchanger.

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DIALOG(R)File 5:Biosis Previews(R)

0012898235 BIOSIS NO.: 200100070074
A single site (Ser16) phosphorylation in %%%phospholamban%%% is sufficient in mediating its maximal cardiac responses to beta-agonists
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JOURNAL: Journal of Biological Chemistry 275 (49): p38938-38943 December 8, 2000 2000
MEDIUM: print
ISSN: 0021-9258
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLB) can be phosphorylated at Ser16 by cyclic AMP-dependent protein kinase and at Thr17 by Ca2+-calmodulin-dependent protein kinase during beta-agonist stimulation. A previous study indicated that %%%mutation%%% of S16A in PLB resulted in lack of Thr17 phosphorylation and attenuation of the beta-agonist stimulatory effects in perfused mouse hearts. To further delineate the functional interplay between dual-site PLB phosphorylation, we generated transgenic mice expressing the T17A mutant PLB in the cardiac compartment of the null background. Lines expressing similar levels of T17A mutant, S16A mutant, or wild-type PLB in the null background were characterized in parallel. Cardiac myocyte basal mechanics and Ca2+ kinetics were similar among the three groups. Isoproterenol stimulation was associated with phosphorylation of both Ser16 and Thr17 in wild-type PLB and Ser16 phosphorylation in T17A mutant PLB, whereas there was no detectable phosphorylation of S16A mutant PLB. Phosphorylation of Ser16 alone in T17A mutant PLB resulted in responses of the mechanical and Ca2+ kinetic parameters to isoproterenol similar to those in wild-type myocytes, which exhibited dual-site PLB phosphorylation. However, those parameters were significantly attenuated in the S16A mutant myocytes. Thus, Ser16 in PLB can be phosphorylated independently of Thr17 in vivo, and phosphorylation of Ser16 is sufficient for mediating the maximal cardiac responses to beta-adrenergic stimulation.

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DIALOG(R)File 5:Biosis Previews(R)

0012799084 BIOSIS NO.: 200000517397
Impaired Ca2+-ATPase oligomerization and increased %%%phospholamban%%% expression in dilated cardiomyopathy
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JOURNAL: International Journal of Molecular Medicine 6 (5): p533-538 November, 2000 2000
MEDIUM: print
ISSN: 1107-3756
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Although primary genetic defects have been identified for some forms of inherited cardiomyopathy, it is not well understood how secondary abnormalities actually lead to muscle cell destruction. Since cardiomyopathies significantly influence morbidity and mortality rates worldwide, it is important to improve the differential diagnosis of these disorders and develop potential treatments for inherited diseases of the heart. Elucidation of the secondary molecular mechanisms underlying cardiac cell necrosis might help linking a specific %%%mutation%%% in a cardiac gene to acute heart failure. As disturbed Ca2+-homeostasis may contribute to heart failure, we have investigated the relative abundance and oligomeric status of the sarcoplasmic reticulum Ca2+-ATPase and %%%phospholamban%%% in various cardiomyopathies. These two proteins represent important factors in cardiac relaxation. The SERCA2 isoform of the Ca2+-ATPase represents a major Ca2+-removal system in cardiac muscle fibres and %%%phospholamban%%% is a regulator of Ca2+-pump activity. Although Ca2+-ATPase expression did not seem to be markedly altered, the comparative immunoblot analysis presented here clearly shows that %%%phospholamban%%% expression is increased in dilated cardiomyopathy, possibly explaining the decreased Ca2+-uptake in the disease. In contrast to the normal enzyme, the Ca2+-pump was demonstrated to exhibit an impairment of crosslinker-stabilized oligomerization in dilated cardiomyopathy. Since Ca2+-ATPase oligomerization is important for co-operative kinetics and protection against proteolytic degradation, the monomeric Ca2+-ATPase may trigger an abnormal contraction-relaxation cycle in dilated cardiomyopathy leading to heart failure.

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DIALOG(R)File 5:Biosis Previews(R)

0012377750 BIOSIS NO.: 200000096063
Characterization of proximal transcription regulatory elements in the rat %%%phospholamban%%% promoter
AUTHOR: McTiernan Charles F (Reprint); Lemster Bonnie H; Frye Carole S; Johns David C; Feldman Arthur M
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JOURNAL: Journal of Molecular and Cellular Cardiology 31 (12): p2137-2153 Dec., 1999 1999
MEDIUM: print
ISSN: 0022-2828
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% is a major regulator of cardiac diastole, with alterations in expression associated with modified cardiac relaxation. To study transcriptional regulation of %%%phospholamban%%% expression, we made reporter constructs that expressed luciferase under control of putative promoter sequences from the rat %%%phospholamban%%% gene. When transfected into neonatal rat cardiomyocytes, constructs containing at least 159 nucleotides preceding the transcription start site were equally active, while truncation to -66/+64 removed all promoter activity. Constructs were more active in cardiomyocytes than in HeLa cells (which do not express %%%phospholamban%%%), but did not show absolute cell-type specificity of expression. Addition of sequences upstream to -4032, all of the intron (7.4 kb), or 3'UTR sequences (0.8 kb) did not enhance cell-specific expression. To focus on the basal promoter region (-159/-66), a series of deletion constructs were made that identified a novel 35 bp region (-159/-125; %%%Phospholamban%%% Promoter Element 1, PPE1) required for promoter activity in cardiomyocytes. Site-specific %%%mutations%%% identified nucleotides -150/-133 as containing most of the promoter-enhancing activity. While the rat PPE1 is highly conserved (>70%) in four other mammalian %%%phospholamban%%% genes, it does not contain previously characterized regulatory elements. In cardiomyocytes the PPE1 sequence markedly enhanced activity of the SV40 early promoter. A conserved CCAAT element (-83/-79) was also required for promoter activity in both cardiomyocytes and HeLa cells. Exonuclease III footprinting identified protein/DNA interactions in both the extended CCAAT box and PPE1 domains. Gel shift studies identified the CCAAT elements as binding CBF/NF-Y.

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DIALOG(R)File 5:Biosis Previews(R)

0012318894 BIOSIS NO.: 200000037207
Ablation of %%%phospholamban%%% phosphorylation sites in transgenic mouse hearts
AUTHOR: Brittsan Angela G (Reprint); Schmidt Albrecht (Reprint); Grupp Ingrid L (Reprint); Hoit Brian D (Reprint); Kranias Evangelia G (Reprint)
AUTHOR ADDRESS: Univ of Cincinnati, Cincinnati, OH, USA**USA
JOURNAL: Circulation 100 (18 SUPPL.): pI.763 Nov. 2, 1999 1999
MEDIUM: print
CONFERENCE/MEETING: 72nd Scientific Sessions of the American Heart Association Atlanta, Georgia, USA November 7-10, 1999; 19991107
ISSN: 0009-7322
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/43
DIALOG(R)File 5:Biosis Previews(R)

0012308555 BIOSIS NO.: 200000026868
Transmembrane helix M6 in sarco(endo)plasmic reticulum Ca2+-ATPase forms a functional interaction site with %%%phospholamban%%%: Evidence for physical interactions at other sites
AUTHOR: Asahi Michio; Kimura Yoshihiro; Kurzydlowski Kazimierz; Tada Michihiko; MacLennan David H (Reprint)
AUTHOR ADDRESS: Banting and Best Dept. of Medical Research, University of Toronto, Charles H. Best Inst., 112 College St., Toronto, ON, M5G 1L6, Canada**Canada
JOURNAL: Journal of Biological Chemistry 274 (46): p32855-32862 Nov. 12, 1999 1999
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In an earlier study (Kimura, Y., Kurzydlowski, K., Tada, M., and MacLennan, D. H. (1997) J. Biol. Chem. 272, 15061-15064), %%%mutation%%% of amino acids on one face of the %%%phospholamban%%% (PLN) transmembrane helix led to loss of PLN inhibition of sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) molecules. This helical face was proposed to form a site of PLN interaction with a transmembrane helix in SERCA molecules. To determine whether predicted transmembrane helices M4, M5, M6, or M8 in SERCA1a interact with PLN, SERCA1a mutants were co-expressed with wild-type PLN and effects on Ca2+ dependence of Ca2+ transport were measured. Wild-type inhibitory interactions shifted apparent Ca2+ affinity of SERCA1a by an average of -0.34 pCa units, but four of the seven %%%mutations%%% in M4 led to a more inhibitory shift in apparent Ca2+ affinity, averaging -0.53 pCa units. Seven %%%mutations%%% in M5 led to an average shift of -0.32 pCa units and seven %%%mutations%%% in M8 led to an average shift of -0.30 pCaunits. Among 11 %%%mutations%%% in M6, 1, Q791A, increased the inhibitory shift (-0.59 pCa units) and 5, V795A (-0.11), L802A (-0.07), L802V (-0.04), T805A (-0.11), and F809A (-0.12), reduced the inhibitory shift, consistent with the view that Val795, Leu802, Thr805, and Phe809, located on one face of a predicted M6 helix, form a site in SERCA1a for interaction with PLN. Those %%%mutations%%% in M4, M6, or M8 of SERCA1a that enhanced PLN inhibitory function did not enhance PLN physical association with SERCA1a, but mutants V795A and L802A in M6, which decreased PLN inhibitory function, decreased physical association, as measured by co-immunoprecipitation. In related studies, those PLN mutants that gained inhibitory function also increased levels of co-immunoprecipitation of wild-type SERCA1a and those that lost inhibitory function also reduced association, correlating functional interaction sites with physical interaction sites. Thus, both functional and physical data confirm that PLNinteracts with M6 SERCA1a.

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DIALOG(R)File 5:Biosis Previews(R)

0012081393 BIOSIS NO.: 199900341053
cAPK-phosphorylation controls the interaction of the regulatory domain of cardiac myosin binding protein C with myosin-S2 in an on-off fashion
AUTHOR: Gruen Mathias; Prinz Heino; Gautel Mathias (Reprint)
AUTHOR ADDRESS: Structural Biology Division, European Molecular Biology Laboratory, 69012, Heidelberg, Germany**Germany
JOURNAL: FEBS Letters 453 (3): p254-259 June 25, 1999 1999
MEDIUM: print
ISSN: 0014-5793
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Myosin binding protein C is a protein of the myosin filaments of striated muscle which is expressed in isoforms specific for cardiac and skeletal muscle. The cardiac isoform is phosphorylated rapidly upon adrenergic stimulation of myocardium by cAMP-dependent protein kinase,

and together with the phosphorylation of troponin-I and %%%phospholamban%%% contributes to the positive inotropy that results from adrenergic stimulation of the heart. Cardiac myosin binding protein C is phosphorylated by cAMP-dependent protein kinase on three sites in a myosin binding protein C specific N-terminal domain which binds to myosin-S2. This interaction with myosin close to the motor domain is likely to mediate the regulatory function of the protein. Cardiac myosin binding protein C is a common target gene of familial hypertrophic cardiomyopathy and most %%%mutations%%% encode N-terminal subfragments of myosin binding protein C. The understanding of the signalling interactions of the N-terminal region is therefore important for understanding the pathophysiology of myosin binding protein C associated cardiomyopathy. We demonstrate here by cosedimentation assays and isothermal titration calorimetry that the myosin-S2 binding properties of the myosin binding protein C motif are abolished by cAMP-dependent protein kinase-mediated trisphosphorylation, decreasing the S2 affinity from a Kd of apprxeq 5 muM to undetectable levels. We show that the slow and fast skeletal muscle isoforms are no cAMP-dependent protein kinase substrates and that the S2 interaction of these myosin binding protein C isoforms is therefore constitutively on. The regulation of cardiac contractility by myosin binding protein C therefore appears to be a 'brake-off' mechanism that will free a specific subset of myosin heads from sterical constraints imposed by the binding to the myosin binding protein C motif.

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DIALOG(R)File 5:Biosis Previews(R)

0012013839 BIOSIS NO.: 199900273499
A fluorescence energy transfer method for analyzing protein oligomeric structure: Application to %%%phospholamban%%%
AUTHOR: Li Ming; Reddy Laxma G; Bennett Roberta; Silva Norberto D Jr; Jones Larry R; Thomas David D (Reprint)
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JOURNAL: Biophysical Journal 76 (5): p2587-2599 May, 1999 1999
MEDIUM: print
ISSN: 0006-3495
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have developed a method using fluorescence energy transfer (FET) to analyze protein oligomeric structure. Two populations of a protein are labeled with fluorescent donor and acceptor, respectively, then mixed at a defined donor/acceptor ratio. A theoretical simulation, assuming random mixing and association among protein subunits in a ring-shaped homo-oligomer, was used to determine the dependence of FET on the number of subunits, the distance between labeled sites on different subunits, and the fraction of subunits remaining monomeric. By measuring FET as a function of the donor/acceptor ratio, the above parameters of the oligomeric structure can be resolved over a substantial range of their values. We used this approach to investigate the oligomeric structure of %%%phospholamban%%% (PLB), a 52-amino acid protein in cardiac sarcoplasmic reticulum (SR). Phosphorylation of PLB regulates the SR Ca-ATPase. Because PLB exists primarily as a homopentamer on sodium dodecyl sulfate polyacrylamide gel electrophoresis, it has been proposed that the pentameric structure of PLB is important for its regulatory function. However, this hypothesis must be tested by determining directly the oligomeric structure of PLB in the lipid membrane. To accomplish this goal, PLB was labeled at Lys-3 in the cytoplasmic domain, with two different amine-reactive donor/acceptor pairs, which gave very similar FET results. In detergent solutions, FET was not observed unless the sample was first boiled to facilitate subunit mixing. In lipid bilayers, FET was observed at 25degreeC without boiling, indicating a dynamic equilibrium among PLB subunits in the membrane. Analysis of the FET data indicated that the dye-labeled PLB is predominantly in oligomers having at least 8 subunits, that 7-23% of the PLB subunits are monomeric, and that the distance between dyes on adjacent PLB subunits is about 10 ANG.

A point %%%mutation%%% of PLB (L37A) that runs as monomer on SDS-PAGE showed no energy transfer, confirming its monomeric state in the membrane. We conclude that FET is a powerful approach for analyzing the oligomeric structure of PLB, and this method is applicable to other oligomeric proteins.

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DIALOG(R)File 5:Biosis Previews(R)

0011926318 BIOSIS NO.: 199900185978
Protein-protein interactions of %%%phospholamban%%% and its mutants as studied by EPR spectroscopy and computer simulation
AUTHOR: Stamm John D (Reprint); Karim Christine (Reprint); Dreytser David (Reprint); Paterlini Germana (Reprint); Jones Larry R; Thomas David D (Reprint)
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JOURNAL: Biophysical Journal 76 (1 PART 2): pA124 Jan., 1999 1999
MEDIUM: print
CONFERENCE/MEETING: Forty-third Annual Meeting of the Biophysical Society Baltimore, Maryland, USA February 13-17, 1999; 19990213
ISSN: 0006-3495
DOCUMENT TYPE: Meeting; Meeting Abstract; Meeting Poster
RECORD TYPE: Citation
LANGUAGE: English

2/7/47
DIALOG(R)File 5:Biosis Previews(R)

0011926305 BIOSIS NO.: 199900185965
Structural and functional studies of the synthetic pentameric transmembrane domain and its monomeric full-length %%%mutations%%%, Ala3-%%%phospholamban%%%
AUTHOR: Marquardt Christopher G (Reprint); Hunter Gregory W (Reprint); Jones Larry R; Thomas David D (Reprint); Karim Christine B (Reprint)
AUTHOR ADDRESS: Dept. of Biochemistry, Univ. of Minnesota, Minneapolis, MN, 55455, USA**USA
JOURNAL: Biophysical Journal 76 (1 PART 2): pA122 Jan., 1999 1999
MEDIUM: print
CONFERENCE/MEETING: Forty-third Annual Meeting of the Biophysical Society Baltimore, Maryland, USA February 13-17, 1999; 19990213
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LANGUAGE: English

2/7/48
DIALOG(R)File 5:Biosis Previews(R)

0011796256 BIOSIS NO.: 199900055916
Pentameric assembly of %%%phospholamban%%% facilitates inhibition of cardiac function in vivo
AUTHOR: Chu Guoxiang; Li Li; Sato Yoji; Harrer Judy M; Kadambi Vivek J; Hoit Brian D; Bers Donald M; Kranias Evangelia G (Reprint)
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JOURNAL: Journal of Biological Chemistry 273 (50): p33674-33680 Dec. 11, 1998 1998
MEDIUM: print
ISSN: 0021-9258
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% has been proposed to coexist as pentamers and

monomers in native sarcoplasmic reticulum membranes. To determine its functional unit in vivo, we reintroduced wild-type (pentameric) or monomeric mutant (C41F) %%%phospholamban%%% in the hearts of %%%phospholamban%%% knockout mice. Transgenic lines, expressing similar levels of mutant or wild-type %%%phospholamban%%%, were identified, and their cardiac phenotypes were characterized in parallel. Sarcoplasmic reticulum Ca2+ transport assays indicated similar decreases in SERCA2 Ca2+ affinity by mutant or wild-type %%%phospholamban%%%. However, the time constants of relaxation and Ca2+ transient decline in isolated cardiomyocytes were diminished to a greater extent by wild-type than mutant %%%phospholamban%%%, even without significant differences in the amplitudes of myocyte contraction and Ca2+ transients between the two groups. Langendorff perfusion also indicated that mutant %%%phospholamban%%% was not capable of depressing the enhanced relaxation parameters of the %%%phospholamban%%% knockout hearts to the same extent as wild-type %%%phospholamban%%%. Moreover, in vivo assessment of mouse hemodynamics revealed a greater depression of cardiac function in wild-type than mutant %%%phospholamban%%% hearts. Thus, the mutant or monomeric form of %%%phospholamban%%% was not as effective in slowing Ca2+ decline or relaxation in cardiomyocytes, hearts, or intact animals as wild-type or pentameric %%%phospholamban%%%. These findings suggest that pentameric assembly of %%%phospholamban%%% is necessary for optimal regulation of myocardial contractility in vivo.

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DIALOG(R)File 5:Biosis Previews(R)

0011503141 BIOSIS NO.: 199800297388
Phosphorylation-induced structural change in %%%phospholamban%%% and its mutants, detected by intrinsic fluorescence
AUTHOR: Li Ming; Cornea Razvan L; Autry Joseph M; Jones Larry R; Thomas David D (Reprint)
AUTHOR ADDRESS: Dep. Biochemistry, Univ. Minn. Med. Sch., Minneapolis, MN 55455, USA**USA
JOURNAL: Biochemistry 37 (21): p7869-7877 May 26, 1998 1998
MEDIUM: print
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have used intrinsic fluorescence to test the hypothesis that phosphorylation induces a conformational change in %%%phospholamban%%% (PLB), a regulatory protein in cardiac sarcoplasmic reticulum (SR). Phosphorylation of PLB, which relieves inhibition of the cardiac Ca-ATPase, has been shown to decrease the mobility of PLB in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDSPAGE). In the present study, we found that this mobility shift depends on the acrylamide concentration in the gel, suggesting that phosphorylation increases the effective Stokes radius. To further characterize this structural change, we performed spectroscopic experiments.under the conditions of SDS-PAGE. CD indicated that phosphorylation at Ser-16 does not change PLB's secondary structure significantly. However, the fluorescence of Tyr-6 in the cytoplasmic domain of PLB changed significantly upon PLB phosphorylation: phosphorylation increased the fluorescence quantum yield and decreased the quenching efficiency by acrylamide, suggesting a local structural change that decreases the solvent accessibility of Tyr-6. A point %%%mutation%%% (L37A) in the transmembrane domain, which disrupts PLB pentamers and produces monomers in SDS -PAGE and in lipid bilayers, showed similar phosphorylation effects on fluorescence, indicating that subunit interactions within PLB are not crucial for the observed conformational change in SDS. When PLB was reconstituted into dioleoylphosphatidylcholine (DOPC) lipid bilayers, similar phosphorylation effects in fluorescence were observed, suggesting that PLB behaves similarly in response to phosphorylation in both detergent and lipid environments. We conclude that phosphorylation induces a structural change within the PLB protomer that decreases the solvent accessibility of Tyr-6. The similarity of this structural change in monomers and pentamers is consistent with models in which the PLB

monomer is sufficient for the phosphorylation-dependent regulation of the Ca-ATPase.

2/7/50
DIALOG(R)File 5:Biosis Previews(R)

0011502791 BIOSIS NO.: 199800297038
%%%Phospholamban%%% domain Ib %%%mutations%%% influence functional interactions with the Ca2+-ATPase isoform of cardiac sarcoplasmic reticulum
AUTHOR: Kimura Yoshihiro; Asahi Michio; Kurzydlowski Kazimierz; Tada Michihiko; Maclennan David H (Reprint)
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JOURNAL: Journal of Biological Chemistry 273 (23): p14238-14241 June 5, 1998 1998
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Alanine-scanning mutagenesis of amino acids 21-30, forming cytoplasmic domain 1b in %%%phospholamban%%% (PLN), revealed that %%%mutation%%% to Ala of Asn27, Gln29, and Asn30 results in gain of inhibitory function. In an earlier study (Kimura, Y., Kurzydlowski, K., Tada, M., and MacLennan, D. H. (1997) J. Biol. Chem. 272,15061-15064), gain of function in PLN transmembrane domain II mutants was correlated with pentamer destabilization, leading to proposals that the PLN monomer is the active inhibitory species, that dissociation of the PLN pentamer is one determinant of PLN inhibitory function and that dissociation of the PLN-cardiac sarco(endo)plasmic Ca2+-ATPase isoform (SERCA2a) complex is a second determinant. Because each of the new domain 1b mutants contained a normal ratio of pentamer to monomer in SDS-polyacrylamide gel electrophoresis, gain of function must have resulted from mechanisms other than destabilization of pentameric structure. Evidence that domain 1b and domain II mutants act through different sites and different mechanisms was provided by a monomeric double mutant, N30A/I40A, in which the enhanced inhibitory function of each single mutant was additive. Evidence for an alteration in stability of the PLN/SERCA2a heterodimer was obtained in a study of double mutant N27A/N34A in which inhibitory function was regained by combining a gain of function, domain Ib %%%mutation%%% with a loss of function domain II %%%mutation%%%. These results support the proposal that PLN inhibition of SERCA2a involves, first, depolymerization of PLN and, second, the formation of inhibitory interactions between monomeric PLN and SERCA2a.

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DIALOG(R)File 5:Biosis Previews(R)

0011479209 BIOSIS NO.: 199800273456
Sarcolipin regulates the activity of SERCA1, the fast-twitch skeletal muscle sarcoplasmic reticulum Ca2+-ATPase
AUTHOR: Odermatt Alex; Becker Stefan; Khanna Vijay K; Kurzydlowski Kazimierz; Leisner Elmi; Pette Dirk; Maclennan David H (Reprint)
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JOURNAL: Journal of Biological Chemistry 273 (20): p12360-12369 May 15, 1998 1998
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The 31-amino acid proteolipid, sarcolipin (SLN), is associated with the fast-twitch skeletal muscle sarcoplasmic reticulum Ca2+-ATPase

(SERCA1). Constructs of human and rabbit SLN and of rabbit SLN with the FLAG epitope at its N terminus (NF-SLN) or its C terminus (SLN-FC) were coexpressed with SERCA1 in HEK-293 T-cells. Immunohistochemistry was used to demonstrate colocalization of NF-SLN and SERCA1 in the endoplasmic reticulum membrane and to demonstrate the cytosolic orientation of the N terminus of SLN. Coexpression of native rabbit SLN or NF-SLN with SERCA1 decreased the apparent affinity of SERCA1 for Ca2+ but stimulated maximal Ca2+ uptake rates (Vmax). The N terminus of SLN is not well conserved among species, and the addition of an N-terminal FLAG epitope did not alter SLN function. Anti-FLAG antibody reversed both the inhibition of Ca2+ uptake by NF-SLN at low Ca2+ concentrations and the stimulatory effect of NF-SLN on Vmax. Addition of the FLAG epitope to the highly conserved C terminus decreased the apparent affinity of SERCA1 for Ca2+ relative to native SLN and decreased Vmax significantly. %%%Mutations%%% in the C-terminal domain showed that this sequence is critical for SLN function. %%%Mutational%%% analysis of the transmembrane helix, together with the additive regulatory effects of coexpression of both SLN and %%%phospholamban%%% (PLN) with SERCA1, provided evidence for different mechanisms of interaction of SLN and PLN with SERCA molecules. Ca2+ uptake rates in sarcoplasmic reticulum vesicles, isolated from rabbit fast-twitch muscle (tibialis anterior) subjected to chronic low frequency stimulation, were reduced by approximately 40% in 3- and 4-day stimulated muscle, with a-marginal increase in apparent affinity of SERCA1 for Ca2+. SERCA1 mRNA and protein levels were unaltered after stimulation. In contrast, SLN mRNA was decreased by 15%, and SLN protein was reduced by 40%. Reduced SLN expression could explain the decrease in SERCA1 activity observed in these muscles and might represent an early functional adaptation to chronic low frequency stimulation.

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DIALOG(R)File 5:Biosis Previews(R)

0011457181 BIOSIS NO.: 199800251428
Using experimental information to produce a model of the transmembrane domain of the ion channel %%%phospholamban%%%
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JOURNAL: Biophysical Journal 74 (3): p1203-1214 March, 1998 1998
MEDIUM: print
ISSN: 0006-3495
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Molecular models of the transmembrane domain of the %%%phospholamban%%% pentamer have been generated by a computational method that uses the experimentally measured effects of systematic single-site %%%mutations%%% as a guiding force in the modeling procedure. This method makes the assumptions that 1) the %%%phospholamban%%% transmembrane domain is a parallel five-helix bundle, and 2) nondisruptive %%%mutation%%% positions are lipid exposed, whereas 3) disruptive or partially disruptive %%%mutations%%% are not. Our procedure requires substantially less computer time than systematic search methods, allowing rapid assessment of the effects of different experimental results on the helix arrangement. The effectiveness of the approach is investigated in test calculations on two helix-dimer systems of known structure. Two independently derived sets of mutagenesis data were used to define the restraints for generating models of %%%phospholamban%%%. Both resulting models are left-handed, highly symmetrical pentamers. Although the overall bundle geometry is very similar in the two models, the orientation of individual helices differs by apprx50degree, resulting in different sets of residues facing the pore. This demonstrates how differences in restraints can have an effect on the model structures generated, and how the violation of these restraints can identify inconsistent experimental data.

2/7/53
DIALOG(R)File 5:Biosis Previews(R)

0011366524 BIOSIS NO.: 199800160771
Transgenic approaches to define the functional role of dual site %%%phospholamban%%% phosphorylation
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JOURNAL: Journal of Biological Chemistry 273 (8): p4734-4739 Feb. 20, 1998 1998
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% is a critical regulator of the sarcoplasmic reticulum Ca2+-ATPase activity and myocardial contractility. Phosphorylation of %%%phospholamban%%% occurs on both Ser16 and Thr17 during isoproterenol stimulation. To determine the physiological significance of dual site %%%phospholamban%%% phosphorylation, we generated transgenic models expressing either wild-type or the Ser16 fwdarw Ala mutant %%%phospholamban%%% in the cardiac compartment of the %%%phospholamban%%% knockout mice. Transgenic lines with similar levels of mutant or wild-type %%%phospholamban%%% were studied in parallel. Langendorff perfusion indicated that the basal hyperdynamic cardiac function of the knockout mouse was reversed to the same extent by reinsertion of either wild-type or mutant %%%phospholamban%%%. However, isoproterenol stimulation was associated with much lower responses in the contractile parameters of mutant %%%phospholamban%%% compared with wild-type hearts. These attenuated responses were due to lack of phosphorylation of mutant %%%phospholamban%%%, assessed in 32P labeling perfusion experiments. The lack of %%%phospholamban%%% phosphorylation in vivo was not due to conversion of Ser16 to Ala, since the %%%mutated%%% %%%phospholamban%%% form could serve as substrate for the calcium-calmodulin-dependent protein kinase in vitro. These findings indicate that phosphorylation of Ser16 is a prerequisite for Thr17 phosphorylation in %%%phospholamban%%%, and prevention of phosphoserine formation results in attenuation of the beta-agonist stimulatory responses in the mammalian heart.

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DIALOG(R)File 5:Biosis Previews(R)

0011211766 BIOSIS NO.: 199800006013
Characterization of the gene encoding human sarcolipin (SLN), a proteolipid associated with SERCA1: Absence of structural %%%mutations%%% in five patients with Brody disease
AUTHOR: Odermatt Alex; Taschner Peter E M; Scherer Stephen W; Beatty Barbara; Khanna Vijay K; Cornblath David R; Chaudhry Vinay; Yee Won-Chee; Schrank Bertold; Karpati George; Breuning Martin H; Knoers Nine; Maclennan David H (Reprint)
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JOURNAL: Genomics 45 (3): p541-553 Nov. 1, 1997 1997
MEDIUM: print
ISSN: 0888-7543
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Sarcolipin (SLN) is a low-molecular-weight protein that copurifies with the fast-twitch skeletal muscle sarcoplasmic reticulum Ca2+ ATPase (SERCA1). Genomic DNA and cDNA encoding human sarcolipin (SLN) were isolated and characterized and the SLN gene was mapped to chromosome 11q22-q23. Human, rabbit, and mouse cDNAs encode a protein of 31 amino acids. Homology of SLN with %%%phospholamban%%% (PLN) suggests that the first 7 hydrophilic amino acids are cytoplasmic, the next 19

hydrophobic amino acids form a single transmembrane helix, and the last 5 hydrophilic amino acids are lumenal. The cytoplasmic and transmembrane sequences are not well conserved among the three species, but the lumenal sequence is highly conserved. Like SERCA1, SLN is highly expressed in rabbit fast-twitch skeletal muscle, but it is expressed to a lower extent in slow-twitch muscle and to an even lower extent in cardiac muscle, where SERCA2a and PLN are highly expressed. It is expressed in only trace amounts in pancreas and prostate. SLN and PLAT genes resemble each other in having two small exons, with their entire coding sequences lying in exon 2 and a large intron separating the two segments. Brody disease is an inherited disorder of skeletal muscle function, characterized by exercise-induced impairment of muscle relaxation. %%%Mutations%%% in the ATP2A1 gene encoding SERCA1 have been associated with the autosomal recessive inheritance of Brody disease in three families, but not with autosomal dominant inheritance of the disease. A search for %%%mutations%%% in the SLN gene in five Brody families, four of which were not linked to ATP2A1, did not reveal any alterations in coding, splice junction or promoter sequences. The homozygous deletion of C438 in the coding sequence of ATP2A1 in Brody disease family 3, leading to a frameshift and truncation following Pro147 in SERCA1, is the fourth ATP2A1 %%%mutation%%% to be associated with autosomal recessive Brody disease.

2/7/55
DIALOG(R)File 5:Biosis Previews(R)

0011152564 BIOSIS NO.: 199799786624
Linkage of familial dilated cardiomyopathy with conduction defect and muscular dystrophy to chromosome 6q23
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JOURNAL: American Journal of Human Genetics 61 (4): p909-917 1997 1997
ISSN: 0002-9297
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Inherited cardiomyopathies may arise from %%%mutations%%% in genes that are normally expressed in both heart and skeletal muscle and therefore may be accompanied by skeletal muscle weakness. Phenotypically, patients with familial dilated cardiomyopathy (FDC) show enlargement of all four chambers of the heart and develop symptoms of congestive heart failure. Inherited cardiomyopathies may also be accompanied by cardiac conduction-system defects that affect the atrioventricular node, resulting in bradycardia. Several different chromosomal regions have been linked with the development of autosomal dominant FDC, but the gene defects in these disorders remain unknown. We now characterize an autosomal dominant disorder involving dilated cardiomyopathy, cardiac conduction-system disease, and adult-onset limb-girdle muscular dystrophy (FDC, conduction disease, and myopathy (FDC-CDM)). Genetic linkage was used to exclude regions of the genome known to be linked to dilated cardiomyopathy and muscular dystrophy phenotypes and to confirm genetic heterogeneity of these disorders. A genomewide scan identified a region on the long arm of chromosome 6 that is significantly associated with the presence of myopathy (D6S262; maximum LOD score (Z-max) 4.99 at maximum recombination fraction (theta-max) .00), identifying FDC-CDM as a genetically distinct disease. Haplotype analysis refined the interval containing the genetic defect, to a 3-cM interval between D6S1705 and D6S1656. This haplotype analysis excludes a number of striated muscle-expressed genes present in this region, including laminin alpha-2, laminin alpha-4, triadin, and %%%phospholamban%%%.

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DIALOG(R)File 5:Biosis Previews(R)

0011149064 BIOSIS NO.: 199799783124
Monomeric %%%phospholamban%%% overexpression in transgenic mouse hearts
AUTHOR: Chu Guoxiang; Ii Gerald W Dorn; Luo Wusheng; Harrer Judy M; Kadambi Vivek J; Walsh Richard A; Kranias Evangelia G (Reprint)
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JOURNAL: Circulation Research 81 (4): p485-492 1997 1997
ISSN: 0009-7330
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%%, a prominent modulator of the sarcoplasmic reticulum (SR) Ca-2+-ATPase activity and basal contractility in the mammalian heart, has been proposed to form pentamers in native SR membranes. However, the monomeric form of %%%phospholamban%%%, which is associated with %%%mutating%%% Cys-41 to Phe-41, was shown to be as effective as pentameric %%%phospholamban%%% in inhibiting Ca-2+ transport in expression systems. To determine whether this monomeric form of %%%phospholamban%%% is also functional in vivo, we generated transgenic mice with cardiac-specific overexpression of the mutant (Cys-41 fwdarw Phe-41) %%%phospholamban%%%. Quantitative immunoblotting indicated a 2-fold increase in the cardiac %%%phospholamban%%% protein levels compared with wild-type controls, with apprxeq 50% of %%%phospholamban%%% migrating as monomers and apprxeq 50% as pentamers upon SDS-PAGE. The mutant-%%%phospholamban%%% transgenic hearts were analyzed in parallel with transgenic hearts overexpressing (2-fold) wild-type %%%phospholamban%%%, which migrated as pentamers upon SDS-PAGE. SR Ca-2+-uptake assays revealed that the EC-50, values for Ca-2+ were as follows: 0.32+-0.01 mu-mol/L in hearts overexpressing monomeric %%%phospholamban%%%, 0.49+-0.05 mu-mol/L in hearts overexpressing wild-type %%%phospholamban%%%, and 0.26+-0.01 mu-mol/L in wild-type control mouse hearts. Analysis of cardiomyocyte mechanics and Ca-2+ kinetics indicated that the inhibitory effects of mutant-%%%phospholamban%%% overexpression (mt) were less pronounced than those of wild-type %%%phospholamban%%% overexpression (ov) as assessed by depression of the following: (1) shortening fraction (25% mt versus 45% ov), (2) rates of shortening (27% mt versus 48% ov), (3) rates of relengthening (25% mt versus 50% ov), (4) amplitude of the Ca-2+ signal (21% mt versus 40% ov), and (5) time for decay of the Ca-2+ signal (25% mt versus 106% ov) compared with control (100%) myocytes. The differences in basal cardiac myocyte mechanics and Ca-2+ transients among the animal groups overexpressing monomeric or wild-type %%%phospholamban%%% and wild-type control mice were abolished upon isoproterenol stimulation. These findings suggest that pentameric assembly of %%%phospholamban%%% is important for mediating its optimal regulatory effects on myocardial contractility in vivo.

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DIALOG(R)File 5:Biosis Previews(R)

0010970338 BIOSIS NO.: 199799604398
%%%Phospholamban%%% inhibitory function is activated by depolymerization
AUTHOR: Kimura Yoshihiro; Kurzydlowski Kazimierz; Tada Michihiko; Maclennan David H (Reprint)
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JOURNAL: Journal of Biological Chemistry 272 (24): p15061-15064 1997 1997
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLN), a homopentameric, integral membrane protein, reversibly inhibits cardiac sarcoplasmic reticulum Ca-2+-ATPase (SERCA2a) activity through intramembrane interactions. Here, alanine-scanning mutagenesis of the PLN transmembrane sequence was used to identify two functional domains on opposite faces of the transmembrane

helix. %%%Mutations%%% in one face diminish inhibitory interactions with transmembrane sequences of SERCA2a, but have relatively little effect on the pentameric state, while %%%mutations%%% in the other face activate inhibitory interactions and enhance monomer formation. Double mutants are monomeric, but loss of inhibitory function is dominant over activation of inhibitory function. These observations support the proposal that the SERCA2a interaction site lies on the helical face which is not involved in pentamer formation. Four highly inhibitory mutants are effectively devoid of pentamer, suggesting that pentameric PLN represents a less active or inactive reservoir that dissociates to provide inhibitory monomeric PLN subunits. A model is presented in which the degree of PLN inhibition of SERCA2a activity is ultimately determined by the concentration of the inhibited PLN monomer cntdot SERCA2a heterodimeric complex. The concentration of this inhibited complex is determined by the dissociation constant for the PLN pentamer (which is %%%mutation%%% -sensitive) and by the dissociation constant for the PLN/SERCA2a heterodimer (which is likely to be %%%mutation%%%-sensitive).

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DIALOG(R)File 5:Biosis Previews(R)

0010832852 BIOSIS NO.: 199799466912
%%%Mutation%%% and phosphorylation change the oligomeric structure of %%%phospholamban%%% in lipid bilayers
AUTHOR: Cornea Razvan L; Jones Larry R (Reprint); Autry Joseph M; Thomas David D (Reprint)
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JOURNAL: Biochemistry 36 (10): p2960-2967 1997 1997
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLB), a 52-residue protein integral to the cardiac sarcoplasmic reticulum, is a key regulator of the Ca pump. PLB has been shown to form pentamers in the denaturing detergent sodium dodecyl sulfate (SDS), but its oligomeric state in the natural environment of the lipid membrane remains unknown. In order to address this issue, we performed electron paramagnetic resonance (EPR) experiments on two types of lipid-reconstituted, recombinant PLB: wild type (WT PLB) and a mutant substituted with alanine at leucine 37 (L37A PLB), whose propensity to oligomerize in SDS is greatly diminished. The lipid used in reconstitution was dioleoylphosphatidylcholine (DOPC) doped with a phospholipid spin-label that detects protein contact. EPR spectroscopy was used to determine the fraction of the total lipid molecules in contact with PLB. Our results show that, in phospholipid bilayers, WT PLB is oligomeric (effective oligomeric size of 3.52 +- 0.71), while L37A PLB is monomeric (effective oligomeric size of 1.15 +- 0.15). Thus, the oligomeric states of these proteins in the lipid membrane are remarkably similar to those in SDS solution. In particular, the point %%%mutation%%% in L37A PLB greatly destabilizes the PLB oligomer. Phosphorylation of PLB by protein kinase A, which has been shown to relieve inhibition of the cardiac Ca pump, changes the lipid-PLB interactions, decreasing the number of lipids restricted by contact with protein. The results are consistent with a phosphorylation-dependent increase of the effective oligomer size of WT PLB from 3.52 to 5.34 and of L37A PLB from 1.15 to 1.91. These phosphorylation effects were abolished in a medium with a high ionic strength. We conclude that the oligomeric states of PLB in lipid membranes are in a dynamic equilibrium that is perturbed by phosphorylation due to reduced electrostatic repulsion among PLB protomers.

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DIALOG(R)File 5:Biosis Previews(R)

0010255794 BIOSIS NO.: 199698723627

A leucine zipper stabilizes the pentameric membrane domain of %%%phospholamban%%% and forms a coiled-coil pore structure
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JOURNAL: Journal of Biological Chemistry 271 (10): p5941-5946 1996 1996
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% is a phosphoprotein regulator of cardiac sarcoplasmic reticulum which is phosphorylated in response to beta-adrenergic stimulation. Previous results have shown that %%%phospholamban%%% forms Ca-2+-selective channels in lipid bilayers. The channel-forming domain has been localized to amino acid residues 26-52, which form a stable pentameric, helical structure. The specific residues responsible for stabilizing the pentameric membrane domain of %%%phospholamban%%% have been identified by %%%mutational%%% analysis. Residues 26-52 were individually %%%mutated%%% to Ala or Phe, and the ability of the resulting mutant to form a pentamer or other oligomer was assessed by SDS-polyacrylamide gel electrophoresis analysis. Replacement of Leu-37, Ile-40, Leu-44, Ile-47, or Leu-51 by Ala prevented pentamer formation, indicating their essential involvement in the oligomeric assembly. The heptad repeats, and 3-4-residue spacing of the essential amino acids suggest that residues 37-52 adopt a pentameric coiled-coil structure stabilized by a leucine zipper motif formed by the close packing of Leu-37, Ile-40, Leu-44, Ile-47, and Leu-51. The resulting symmetric structure contains a central pore defined by the hydrophobic surface of the five stabilizing leucine zippers, which are oriented to the interior and form the backbone of the pentamer.

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DIALOG(R)File 5:Biosis Previews(R)

0009563866 BIOSIS NO.: 199598031699
Structural organization of the pentameric transmembrane alpha-helices of %%%phospholamban%%%, a cardiac ion channel
AUTHOR: Arkin Isaiah T; Adams Paul D; MacKenzie Kevin R; Lemmon Mark A; Brunger Axel T; Engelman Donald M (Reprint)
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JOURNAL: EMBO (European Molecular Biology Organization) Journal 13 (20): p 4757-4764 1994 1994
ISSN: 0261-4189
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% is a 52 amino acid calcium regulatory protein found as pentamers in cardiac SR membranes. The pentamers form through interactions between its transmembrane domains, and are stable in SDS. We have employed a saturation mutagenesis approach to study the detailed interactions between the transmembrane segments, using a chimeric protein construct in which staphylococcal nuclease (a monomeric soluble protein) is fused to the N-terminus of %%%phospholamban%%%. The chimera forms pentamers observable in SDS-PAGE, allowing the effects of %%%mutations%%% upon the oligomeric association to be determined by electrophoresis. The disruptive effects of amino acid substitutions in the transmembrane domain were classified as sensitive, moderately sensitive or insensitive. Residues of the same class lined up on faces of a 3.5 amino acids/turn helical projection, allowing the construction of a model of the interacting surfaces in which the helices are associated in a left-handed pentameric coiled-coil configuration. Molecular modeling simulations (to be described elsewhere in detail) confirm that the helices readily form a left-handed coiled-coil helical bundle and have yielded molecular models for the interacting surfaces, the best of which is identical to that predicted by the mutagenesis. Residues lining the pore show considerable

structural sensitivity to %%%mutation%%%, indicating that care must be taken in interpreting the results of mutagenesis studies of channels. The cylindrical ion pore (minimal diameter of 2 ANG) appears to be defined largely by hydrophobic residues (140, L43 and 147) with only two mildly polar elements contributed by sulfurs in residues C36 and M50.

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DIALOG(R)File 5:Biosis Previews(R)

0009470982 BIOSIS NO.: 199497492267
Amino acids Lys-Asp-Asp-Lys-Pro-Val-402 in the Ca-2+-ATPase of cardiac sarcoplasmic reticulum are critical for functional association with %%%phospholamban%%%
AUTHOR: Toyofuku Toshihiko; Kurzydlowski Kazimierz; Tada Michihiko; Maclennan David H (Reprint)
AUTHOR ADDRESS: Banting Best Dep. Med. Res., Univ. Toronto, Charles H. Best Inst., 112 College St., Toronto, ON M5G 1L6, Canada**Canada
JOURNAL: Journal of Biological Chemistry 269 (37): p22929-22932 1994 1994
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% interacts with SERCA2 (sarco/endoplasmic reticulum calcium ATPase isoform 2) but not SERCA3. The use of chimeric SERCA2/SERCA3 molecules has revealed that amino acids 336-412 in the phosphorylation domain of SERCA2 are essential for functional association with %%%phospholamban%%% (Toyofuku, T., Kurzycllowski, K., Tada, M., and MacLennan, D. H. (1993) J. Biol. Chem. 268, 2809-2815). When %%%mutations%%% were made in SERCA2 between amino acids 336 and 412 and the mutants were coexpressed with %%%phospholamban%%%, only %%%mutation%%% of amino acids Lys-397 to Val-402 affected %%%phospholamban%%% association with the Ca-2+-ATPase. A chimeric Ca-2+-ATPase, CH2, was created in which the phosphorylation domain of SERCA2 was replaced with that of SERCA3, disrupting functional interaction with %%%phospholamban%%%. The SERCA3 sequence QGEQLV-402 was then %%%mutated%%% to the corresponding SERCA2 sequence, KDDKPV-402, and to the sequence KGEKPV-402, resulting in restoration of functional interaction with %%%phospholamban%%%. %%%Mutation%%% to KGNKPV-402 or to QGEQPV-402 did not restore functional interaction with %%%phospholamban%%%. These results demonstrate that amino acids Lys-397-Val-402 comprise the interaction site with %%%phospholamban%%% in SERCA2, and probably also in SERCA1, and that the appropriate balance of charged and hydrophobic residues is an important feature of the interaction.

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DIALOG(R)File 5:Biosis Previews(R)

0009153837 BIOSIS NO.: 199497175122
Amino Acids Glu-2 to Ile-18 in the Cytoplasmic Domain of %%%Phospholamban%%% Are Essential for Functional Association with the Ca-2+-ATPase of Sarcoplasmic Reticulum
AUTHOR: Toyofuku Toshihiko; Kurzydlowski Kazimierz; Tada Michihiko; Maclennan David H (Reprint)
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JOURNAL: Journal of Biological Chemistry 269 (4): p3088-3094 1994 1994
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% inhibits the Ca-2+-ATPase of cardiac sarcoplasmic reticulum by lowering its affinity for Ca-2+. In earlier studies (Toyofuku, T., Kazimierz, K., Tada, M., and MacLennan, D. H. (1993) J. Biol. Chem. 268, 2809-2815), parts of the phosphorylation and

nucleotide binding/hinge domains of the Ca-2+-ATPase were shown to be essential for %%%phospholamban%%% interaction. In order to identify the sites in %%%phospholamban%%% which interact with the Ca-2+-ATPase, a series of mutants containing amino acid replacements in the cytoplasmic and transmembrane regions of %%%phospholamban%%% were co-expressed with the cardiac/slow-twitch muscle Ca-2+-ATPase isozyme, SERCA2a, in HEK-293 cells. %%%Mutation%%% of residues in the cytoplasmic 1A domain of %%%phospholamban%%%, including positively charged residues, Lys-3, Arg-9, Arg-13, and Arg-14, negatively charged residue, Glu-2, hydrophobic residues, Val-4, Leu-7, Ala-11, Ile-12, Ala-15, and Ile-18, and phosphorylation site residues, Ser-16 and Thr-17, resulted in loss of the inhibitory effect of %%%phospholamban%%% on Ca-2+ transport by the Ca-2+-ATPase. By contrast, %%%mutation%%% of Met-1, Gln-5, Tyr-6, Thr-8, Ser-10, Glu-19, or Met-20 or of residues in the cytoplasmic 1B domain (Pro-21 to Asn-30 and of Cys-41 in the transmembrane domain (Leu-31 to Leu-52) did not diminish the inhibitory effects of %%%phospholamban%%% on Ca-2+ transport. These results suggest that a region essential for functional association of %%%phospholamban%%% with the Ca-2+-ATPase lies in the cytoplasmic 1A domain of %%%phospholamban%%%.

2/7/63
DIALOG(R)File 5:Biosis Previews(R)

0008886876 BIOSIS NO.: 199396051292
Cloning of calcium-ATPase gene of Plasmodium falciparum and comparison with vertebrate calcium-ATPases
AUTHOR: Kimura Masatsugu (Reprint); Yamaguchi Yoshiko; Takada Suehisa; Tanabe Kazuyuki
AUTHOR ADDRESS: Biophys. Lab., Osaka City Univ. Med. Sch., Asahi-machi, Abeno-ku, Osaka 545, Japan**Japan
JOURNAL: Journal of Cell Science 104 (4): p1129-1136 1993
ISSN: 0021-9533
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A Ca-2+-ATPase gene was cloned from the genomic libraries of Plasmodium falciparum. From the deduced amino acid sequence of the gene, a 139 kDa protein with a total of 1228 amino acids was predicted. Sequence of a partial cDNA clone of the gene identified two introns near the 3'-end at the regions identical to the regions assumed for the Ca-2+-ATPase gene of P. yoelii, a rodent malaria species. As compared with a variety of Ca-2+-ATPases, the P. falciparum Ca-2+-ATPase had the highest amino acid sequence homology (78%) to the P. yoelii Ca-2+-ATPase, moderate homology (45-50%) to vertebrate sarcoplasmic/endoplasmic reticulum Ca-2+-ATPases (SERCAs) and lowest homology (20%) to a plasma membrane Ca-2+-ATPase. The P. falciparum protein conserved sequences and residues that are important for the function and/or structure of the organellar type Ca-2+-ATPase, such as high affinity Ca-2+-binding sites, fluorescein isothiocyanate (FITC)-binding regions, and the phosphorylation site, but the protein did not contain calmodulin-binding regions that occur in the plasma membrane type Ca-2+-ATPase. Thus we concluded the cloned gene was the organellar type Ca-2+-ATPase of P. falciparum. In a region between the phosphorylation site and FITC-binding region, the P. falciparum protein was about 200 residues longer than the rabbit SERCA and lacked a sequence that binds to %%%phospholamban%%%, a protein that regulates the activity of the rabbit SERCA. Comparison of the two malarial Ca-2+-ATPases with site-directed mutants of the rabbit SERCA showed perfect conservation of residues, the %%%mutations%%% of which resulted in dysfunction of the enzyme, but low conservation of residues, the %%%mutations%%% of which affected enzyme activity little. Incorporation of the malarial sequences into wheel diagrams for analysis of 10 transmembrane domains of the organellar Ca-2+-ATPase showed a cluster of conserved residues on the surface of each alpha-helix. Our comparison suggest that the malarial Ca-2+-ATPases are useful for identifying residues or regions that are important for the function and/or structure of the organellar Ca-2+-ATPase.

2/7/64
DIALOG(R)File 5:Biosis Previews(R)

0007735903 BIOSIS NO.: 199191118794
CALCIUM HOMEOSTASIS AND TRANSPORT ARE AFFECTED BY DISRUPTION OF CTA3 A NOVEL GENE ENCODING CALCIUM ATPASE IN SCHIZOSACCHAROMYCES-POMBE
AUTHOR: GHISLAIN M (Reprint); GOFFEAU A; HALACHMI D; EILAM Y
AUTHOR ADDRESS: UNITE DE BIOCHIMIE PHYSIOLOGIQUE, PLACE CROIX DU SUD 2/20, 1348 LOUVAIN-LA-NEUVE, BELGIUM**BELGIUM
JOURNAL: Journal of Biological Chemistry 265 (30): p18400-18407 1990
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A new P-type ATPase gene cta3, has been identified in Schizosaccharomyces pombe. The deduced amino acid sequence presents a 45% identity with the Saccharomyces cerevisiae putative Ca2+-ATPase encoded by the PMR2 gene. The cta3 protein contains 7 out of 8 amino acid residues involved in high affinity Ca2+ binding in the sarcoplasmic reticulum Ca2+-ATPase from muscles. It also contains a region similar to the %%%phospholamban%%%-binding domain that characterizes this Ca2+ pump. A null %%%mutation%%% of cta3 leads to higher levels of cytosolic free Ca2+ and to lower amounts of sequestered and bound Ca2+. Cellular Ca2+ efflux and rates of uptake into intracellular compartments are reduced by the loss of cta3 function. The sequence analysis and the physiological results strongly support the conclusion that the cta3 gene encodes a Ca2+-ATPase, probably located in intracellular membranes.

2/7/65
DIALOG(R)File 5:Biosis Previews(R)

0006766178 BIOSIS NO.: 198988081293
EXPRESSION AND SITE-SPECIFIC MUTAGENESIS OF %%%PHOSPHOLAMBAN%%% STUDIES OF RESIDUES INVOLVED IN PHOSPHORYLATION AND PENTAMER FORMATION
AUTHOR: FUJII J (Reprint); MARUYAMA K; TADA M; MACLENNAN D H
AUTHOR ADDRESS: BANTING AND BEST DEP MED RES, CHARLES H BEST INST, UNIV TORONTO, TORONTO, ONTARIO M5G 1L6, CANADA**CANADA
JOURNAL: Journal of Biological Chemistry 264 (22): p12950-12955 1989
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Full-length cDNAs encoding either dog cardiac or rabbit skeletal muscle %%%phospholamban%%% were expressed transiently in COS-1 cells. The expressed protein displayed the mobility of a pentamer when dissolved in sodium dodecyl sulfate and separated in polyacrylamide gels, and of a monomer when boiled prior to polyacrylamide gel separation. Site-specific mutagenesis was used to analyze the roles of several amino acids in the structure and function of the protein. Ser16 and Thr17 were shown to be phosphorylated uniquely by cAMP- and calmodulin-dependent protein kinases, respectively, confirming earlier observations on the native protein (Simmerman, H. K. B., Collins, J. H., Theibert, J. L., Wegener, A. D., and Jones, L. R. (1986) J. Biol. Chem. 261, 13333-13341). Arg13 and Arg14 were shown to be essential for both types of phosphorylation, and Arg9 was shown to be essential for calmodulin-dependent phosphorylation. In studies of pentamer stability, %%%mutation%%% of Gln22-Gln23 to Ala-Ala or Glu-Glu, of Gln26-Asn27 to Glu-Asp, or of Gln29-Asn30 to Glu-Asp had no effect on thermal stability of the pentamer, suggesting that hydrogen bonding involving these residues in domain IB is not important for pentamer stability. By contrast, %%%mutation%%% of Cys36, Cys41, and Cys46 in transmembrane domain II to Ser, Ala, or Phe diminished the stability of the pentamer when microsomal proteins were dissociated in sodium dodecyl sulfate and separated by polyacrylamide gel electrophoresis. In particular, the Cys41 to Phe mutant existed as a monomer at ambient temperature. These results suggest that the intramembranous cysteine residues are important for pentamer

formation even though they are not disulfide-bonded.

2/7/66
DIALOG(R)File 5:Biosis Previews(R)

0005953692 BIOSIS NO.: 198835050797
SITE-SPECIFIC %%%MUTATION%%% OF THE CALCIUM ATPASE OF SARCOPLASMIC RETICULUM
AUTHOR: MARUYAMA K (Reprint); FUJII J; TADA M; MACLENNAN D H
AUTHOR ADDRESS: BANTING AND BEST DEP MED RES, UNIV TORONTO, TORONTO, CAN M5G 1L6**CANADA
JOURNAL: Journal of Cellular Biochemistry Supplement (12 PART C): p309 1988
CONFERENCE/MEETING: MEETING ON CELLULAR AND MOLECULAR BIOLOGY OF MUSCLE DEVELOPMENT HELD AT THE UCLA (UNIVERSITY OF CALIFORNIA-LOS ANGELES) SYMPOSIUM ON MOLECULAR AND CELLULAR BIOLOGY, FEBRUARY 28-APRIL 10, 1988. J CELL BIOCHEM SUPPL.
ISSN: 0733-1959
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH

? t s7/7/1-21

7/7/1
DIALOG(R)File 5:Biosis Previews(R)

0014882323 BIOSIS NO.: 200400253080
Effects of phospholipids on the oligomeric structure of %%%phospholamban%%% (PLN), a regulator of Ca2+-ATPase of cardiac sarcoplasmic reticulum (SR).
AUTHOR: Zhang Xiaoming (Reprint); %%%Kimura Yoshihiro%%% (Reprint); Inui Makoto (Reprint
AUTHOR ADDRESS: Dept. Pharmacol, Yamaguchi Univ. Sch. Med, Ube, 755-8505, Japan**Japan
JOURNAL: Journal of Pharmacological Sciences 94 (Supplement 1): p109P 2004 2004
MEDIUM: print
CONFERENCE/MEETING: 77th Annual Meeting of the Japanese Pharmacological Society Osaka, Japan March 08-10, 2004; 20040308
SPONSOR: Japanese Pharmacological Society
ISSN: 1347-8613 _(ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

7/7/2
DIALOG(R)File 5:Biosis Previews(R)

0013661138 BIOSIS NO.: 200200254649
A screening system of novel inotropic agents which modify %%%phospholamban%%%-SERCA interactions
AUTHOR: %%%Kimura Yoshihiro%%% (Reprint); Inui Makoto (Reprint
AUTHOR ADDRESS: Department of Pharmacology, Yamaguchi University School of Medicine, Ube, Yamaguchi, 755-8505, Japan**Japan
JOURNAL: Japanese Journal of Pharmacology 88 (Supplement 1): p262P 2002 2002
MEDIUM: print
CONFERENCE/MEETING: 75th Annual Meeting of the Japanese Pharmacological Society Kumamoto, Japan March 13-15, 2002; 20020313
SPONSOR: Japanese Pharmacological Society
ISSN: 0021-5198
DOCUMENT TYPE: Meeting; Meeting Abstract; Meeting Poster
RECORD TYPE: Citation
LANGUAGE: English

7/7/3
DIALOG(R)File 5:Biosis Previews(R)

0013606026 BIOSIS NO.: 200200199537
Reconstitution of the cytoplasmic interaction between %%%phospholamban%%% and Ca2+-ATPase of cardiac sarcoplasmic reticulum
AUTHOR: %%%Kimura Yoshihiro%%%; Inui Makoto (Reprint
AUTHOR ADDRESS: Department of Pharmacology, Yamaguchi University School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi, 755-8505, Japan**Japan
JOURNAL: Molecular Pharmacology 61 (3): p667-673 March, 2002 2002
MEDIUM: print
ISSN: 0026-895X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLN) reversibly inhibits the Ca2+-ATPase of cardiac sarcoplasmic reticulum (SERCA2a) through a direct protein-protein interaction, playing a pivotal role in the regulation of intracellular Ca2+ in heart muscle cells. The interaction between PLN and SERCA2a occurs at multiple sites within the cytoplasmic and membrane domains. Here, we have reconstituted the cytoplasmic protein-protein interaction using bacterially expressed fusion proteins of the cytoplasmic domain of PLN and the long cytoplasmic loop of SERCA2a. We have developed two methods to evaluate the binding of the fusion proteins, one with glutathione-Sepharose beads and the other with a 96-well plate. Essentially the same results were obtained by the two methods. The affinity of the binding (KD) was 0.70 muM. The association was inhibited by cAMP-dependent phosphorylation of the PLN fusion protein and by usage of anti-PLN monoclonal antibody. It was also diminished by substitution at the phosphorylation site of PLN of Ser16 to Asp. These results suggest that PLN can bind SERCA2a in the absence of the membrane domains and that the modifications of the cytoplasmic domain of PLN that activate SERCA2a parallel the disruption of the association between the two fusion proteins. It has been shown that the removal of PLN inhibition of SERCA2a rescues cardiac function and morphology in the mouse dilated cardiomyopathy model. Our assay system can be applied to the screening of novel inotropic agents that remove the inhibition of SERCA2a by PLN, improving the relaxation as well as the contractility of the failing heart.

7/7/4
DIALOG(R)File 5:Biosis Previews(R)

0013218601 BIOSIS NO.: 200100390440
Superinhibition of sarcoplasmic reticulum function by %%%phospholamban%%% induces cardiac contractile failure
AUTHOR: Haghighi Kobra; Schmidt Albrecht G; Hoit Brian D; Brittsan Angela G ; Yatani Atsuko; Lester James W; Zhai Jing; %%%Kimura Yoshihiro%%%; Dorn Gerald W II; MacLennan David H; Kranias Evangelia G (Reprint
AUTHOR ADDRESS: Dept. of Pharmacology and Cell Biophysics, University of Cincinnati, College of Medicine, 231 A. Sabin Way, Cincinnati, OH, 45267, USA**USA
JOURNAL: Journal of Biological Chemistry 276 (26): p24145-24152 June 29, 2001 2001
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: To determine whether selective impairment of cardiac sarcoplasmic reticulum (SR) Ca2+ transport may drive the progressive functional deterioration leading to heart failure, transgenic mice, overexpressing a %%%phospholamban%%% Val49 fwdarw Gly mutant (2-fold), which is a superinhibitor of SR Ca2+-ATPase affinity for Ca2+, were generated, and their cardiac phenotype was examined longitudinally. At 3 months of age, the increased EC50 level of SR Ca2+ uptake for Ca2+ (0.67 +- 0.09 muM)

Val49 Ala = loss of function

resulted in significantly higher depression of cardiomyocyte rates of shortening (57%), relengthening (31%), and prolongation of the Ca2+ signal decay time (165%) than overexpression (2-fold) of wild type %%%phospholamban%%% (68%, 64%, and 125%, respectively), compared with controls (100%). Echocardiography also revealed significantly depressed function and impaired beta-adrenergic responses in mutant hearts. The depressed contractile parameters were associated with left ventricular remodeling, recapitulation of fetal gene expression, and hypertrophy, which progressed to dilated cardiomyopathy with interstitial tissue fibrosis and death by 6 months in males. Females also had ventricular hypertrophy at 3 months but exhibited normal systolic function up to 12 months of age. These results suggest a causal relationship between defective SR Ca2+ cycling and cardiac remodeling leading to heart failure, with a gender-dependent influence on the time course of these alterations.

7/7/5
DIALOG(R)File 5:Biosis Previews(R)

0013027305 BIOSIS NO.: 200100199144
Physical association between the cytoplasmic domains of the Ca2+-ATPase of cardiac sarcoplasmic reticulum and %%%phospholamban%%%
AUTHOR: %%%Kimura Yoshihiro%%% (Reprint); Inui Makoto (Reprint
AUTHOR ADDRESS: Dept. of Pharmacology, Yamaguchi Univ. Sch. Medicine, Ube, 755-8505, Japan**Japan
JOURNAL: Japanese Journal of Pharmacology 85 (Supplement 1): p77P 2001 2001
MEDIUM: print
CONFERENCE/MEETING: 74th Annual Meeting of the Japanese Pharmacological Society Yokohama, Japan March 21-23, 2001; 20010321
SPONSOR: Japanese Pharmacological Society
ISSN: 0021-5198
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

7/7/6
DIALOG(R)File 5:Biosis Previews(R)

0012855832 BIOSIS NO.: 200100027671
Physical association between the cytoplasmic domains of the Ca2+-ATPase of cardiac sarcoplasmic reticulum and %%%phospholamban%%%
AUTHOR: %%%Kimura Yoshihiro%%% (Reprint); Inui Makoto (Reprint
AUTHOR ADDRESS: Department of Pharmacology, Yamaguchi University School of Medicine, Ube, Yamaguchi, Japan**Japan
JOURNAL: Journal of Molecular and Cellular Cardiology 32 (11): pA112 November, 2000 2000
MEDIUM: print
CONFERENCE/MEETING: XVII Annual Meeting of the International Society for Heart Research, Japanese Section Osaka, Japan December 06-08, 2000; 20001206
SPONSOR: International Society for Heart Research
ISSN: 0022-2828
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

7/7/7
DIALOG(R)File 5:Biosis Previews(R)

0012641281 BIOSIS NO.: 200000359594
The transgenic expression of highly inhibitory monomeric forms of %%%phospholamban%%% in mouse heart impairs cardiac contractility
AUTHOR: Zvaritch Elena; Backx Peter H; Jirik Frank; %%%Kimura Yoshihiro%%%; de Leon Stella; Schmidt Albrecht G; Hoit Brian D; Lester J William;

Kranias Evangelia G; MacLennan David H (Reprint
AUTHOR ADDRESS: Banting and Best Department of Medical Research, University of Toronto, Charles H. Best Institute, 112 College St., Toronto, ON, M5G 1L6, Canada**Canada
JOURNAL: Journal of Biological Chemistry 275 (20): p14985-14991 May 19, 2000 2000
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Transgenic mice were generated with cardiac-specific overexpression of the monomeric, dominant-acting, superinhibitory L37A and I40A mutant forms of %%%phospholamban%%% (PLN), and their phenotypes were compared with wild-type (wt) mice or 2-fold overexpressors of wt PLN (wtOE). The level of PLN monomer in cardiac microsomes was increased 11-13-fold, and the apparent affinity of the sarco(endo)plasmic reticulum Ca2+-ATPase for Ca2+ was decreased from pCa 6.22 in wt or 6.12 in wtOE to 5.81 in L37A and 5.72 in I40A. Basal physiological parameters, measured in isolated myocytes, indicated a significant reduction in the rates of shortening (+dL/dt) and relengthening (-dL/dt). Hemodynamic measurements indicated that peak systolic pressure was unaffected but that pressure changes (+dP/dt and -dP/dt) were lowered significantly in both mutant lines, and relaxation time (tau) was also lengthened significantly. Echocardiography for both mutants showed depressed systolic function and an increase in left ventricular mass of over 1.4-fold. Significant decreases in left ventricular shortening fraction and velocity of circumferential shortening and increases in ejection time were corrected by isoproterenol. The use of antibodies specific against Ser16- and Thr17-PLN peptides showed that phosphorylation of both pentameric and monomeric PLN were increased between 1.2- and 2.4-fold in both the L37A and I40A lines but not in the wtOE line. These observations show that overexpression of superinhibitory mutant forms of PLN causes depression of contractile parameters with induction of cardiac hypertrophy, as assessed with echo-cardiography.

7/7/8
DIALOG(R)File 5:Biosis Previews(R)

0012626916 BIOSIS NO.: 200000345229
Cardiac-specific overexpression of a superinhibitory pentameric %%%phospholamban%%% mutant enhances inhibition of cardiac function in vivo
AUTHOR: Zhai Jing; Schmidt Albrecht G; Hoit Brian D; %%%Kimura Yoshihiro%%% ; MacLennan David H; Kranias Evangelia G (Reprint
AUTHOR ADDRESS: Dept. of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, 231 Bethesda Ave., Cincinnati, OH, 45267-0575, USA**USA
JOURNAL: Journal of Biological Chemistry 275 (14): p10538-10544 April 7, 2000 2000
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% is a regulator of the Ca2+ affinity of the cardiac sarcoplasmic reticulum Ca2+ ATPase (SERCA2a) and of cardiac contractility. In vitro expression studies have shown that several mutant %%%phospholamban%%% monomers are superinhibitory, suggesting that monomeric %%%phospholamban%%% is the active species. However, a %%%phospholamban%%% Asn27 fwdarw Ala (N27A) mutant, which maintained a normal pentamer to monomer ratio, was shown to act as a superinhibitor of SERCA2a Ca2+ affinity. To determine whether the pentameric N27A mutant is superinhibitory in vivo, transgenic mice with cardiac-specific overexpression of mutant %%%phospholamban%%% were generated. Quantitative immunoblotting revealed a 61 +- 6% increase in total %%%phospholamban%%% in mutant hearts, with 90% of the overexpressed protein being pentameric.

The EC50 value for Ca2+ dependence of Ca2+ uptake was 0.69 +- 0.07 muM in mutant hearts, compared with 0.29 +- 0.02 muM in wild-type hearts or 0.43 +- 0.03 muM in hearts overexpressing wild-type PLB by 2-fold. Myocytes from %%%phospholamban%%% N27A mutant hearts also exhibited more depressed contractile parameters than wild-type %%%phospholamban%%% overexpressing cells. The shortening fraction was 52%, rates of shortening and relengthening were 46% and 38% respectively, and time for 80% decay of the Ca2+ signal was 146%, compared with wild-types (100%). Langendorff-perfused mutant hearts also demonstrated depressed contractile parameters. Furthermore, in vivo echocardiography showed a depression in the ratio of early to late diastolic transmitral velocity and a 79% prolongation of the isovolumic relaxation time. Isoproterenol stimulation did not fully relieve the depressed contractile parameters at the cellular, organ, and intact animal levels. Thus, pentameric %%%phospholamban%%% N27A mutant can act as a superinhibitor of the affinity of SERCA2a for Ca2+ and of cardiac contractility in vivo.

7/7/9
DIALOG(R)File 5:Biosis Previews(R)

0012504004 BIOSIS NO.: 200000222317
Physical association between the cytoplasmic domains of the Ca2+-ATPase of cardiac sarcoplasmic reticulum and %%%phospholamban%%%
AUTHOR: %%%Kimura Yoshihiro%%% (Reprint); Inui Makoto (Reprint
AUTHOR ADDRESS: Department of Pharmacology, Yamaguchi University School of Medicine, Ube, Yamaguchi, 755-8505, Japan**Japan
JOURNAL: Japanese Journal of Pharmacology 82 (Suppl. 1): p198P 2000 2000
MEDIUM: print
CONFERENCE/MEETING: 73rd Annual Meeting of the Japanese Pharmacological Society. Yokohama, Japan March 23-25, 2000; 20000323
ISSN: 0021-5198
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

7/7/10
DIALOG(R)File 5:Biosis Previews(R)

0012318882 BIOSIS NO.: 200000037195
Cardiac overexpression of a mutant form of %%%phospholamban%%% induces fetal genes, hypertrophy and contractile failure in mice
AUTHOR: Haghighi Kobra (Reprint); Hoit Brian D (Reprint); Schmidt Albrecht (Reprint); Yatani Atsuko (Reprint); %%%Kimura Yoshihiro%%%; Brittsan Angela G; MacLennan David H; Kranias Evangelia G
AUTHOR ADDRESS: Univ of Cincinnati, Cincinnati, OH, USA**USA
JOURNAL: Circulation 100 (18 SUPPL.): pI.761 Nov. 2, 1999 1999
MEDIUM: print
CONFERENCE/MEETING: 72nd Scientific Sessions of the American Heart Association Atlanta, Georgia, USA November 7-10, 1999; 19991107
ISSN: 0009-7322
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

7/7/11
DIALOG(R)File 5:Biosis Previews(R)

0012308555 BIOSIS NO.: 200000026868
Transmembrane helix M6 in sarco(endo)plasmic reticulum Ca2+-ATPase forms a functional interaction site with %%%phospholamban%%%: Evidence for physical interactions at other sites
AUTHOR: Asahi Michio; %%%Kimura Yoshihiro%%%; Kurzydlowski Kazimierz; Tada Michihiko; MacLennan David H (Reprint
AUTHOR ADDRESS: Banting and Best Dept. of Medical Research, University of Toronto, Charles H. Best Inst., 112 College St., Toronto, ON, M5G 1L6,

Canada**Canada
JOURNAL: Journal of Biological Chemistry 274 (46): p32855-32862 Nov. 12, 1999 1999
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In an earlier study (Kimura, Y., Kurzydlowski, K., Tada, M., and MacLennan, D. H. (1997) J. Biol. Chem. 272, 15061-15064), mutation of amino acids on one face of the %%%phospholamban%%% (PLN) transmembrane helix led to loss of PLN inhibition of sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) molecules. This helical face was proposed to form a site of PLN interaction with a transmembrane helix in SERCA molecules. To determine whether predicted transmembrane helices M4, M5, M6, or M8 in SERCA1a interact with PLN, SERCA1a mutants were co-expressed with wild-type PLN and effects on Ca2+ dependence of Ca2+ transport were measured. Wild-type inhibitory interactions shifted apparent Ca2+ affinity of SERCA1a by an average of -0.34 pCa units, but four of the seven mutations in M4 led to a more inhibitory shift in apparent Ca2+ affinity, averaging -0.53 pCa units. Seven mutations in M5 led to an average shift of -0.32 pCa units and seven mutations in M8 led to an average shift of -0.30 pCaunits. Among 11 mutations in M6, 1, Q791A, increased the inhibitory shift (-0.59 pCa units) and 5, V795A (-0.11), L802A (-0.07), L802V (-0.04), T805A (-0.11), and F809A (-0.12), reduced the inhibitory shift, consistent with the view that Val795, Leu802, Thr805, and Phe809, located on one face of a predicted M6 helix, form a site in SERCA1a for interaction with PLN. Those mutations in M4, M6, or M8 of SERCA1a that enhanced PLN inhibitory function did not enhance PLN physical association with SERCA1a, but mutants V795A and L802A in M6, which decreased PLN inhibitory function, decreased physical association, as measured by co-immunoprecipitation. In related studies, those PLN mutants that gained inhibitory function also increased levels of co-immunoprecipitation of wild-type SERCA1a and those that lost inhibitory function also reduced association, correlating functional interaction sites with physical interaction sites. Thus, both functional and physical data confirm that PLNinteracts with M6 SERCA1a.

7/7/12
DIALOG(R)File 5:Biosis Previews(R)

0012306508 BIOSIS NO.: 200000024821
Transmembrane helix M6 in sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) forms a functional interaction site with %%%phospholamban%%%
AUTHOR: Asahi Michio (Reprint); %%%Kimura Yoshihiro%%%; Kurzydlowski Kazimierz; Tada Michihiko; MacLennan David H
AUTHOR ADDRESS: Univ of Toronto, Toronto, ON, Canada**Canada
JOURNAL: Circulation 100 (18 SUPPL.): pI.421 Nov. 2, 1999 1999
MEDIUM: print
CONFERENCE/MEETING: 72nd Scientific Sessions of the American Heart Association Atlanta, Georgia, USA November 7-10, 1999; 19991107
ISSN: 0009-7322
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

7/7/13
DIALOG(R)File 5:Biosis Previews(R)

0012013905 BIOSIS NO.: 199900273565
Mechanism of %%%phospholamban%%% inhibition of SERCA2a
AUTHOR: %%%Kimura Yoshihiro%%% (Reprint); Inui Makoto (Reprint); Asahi Michio; MacLennan David H; Tada Michihiko
AUTHOR ADDRESS: Yamaguchi Univ. School of Medicine, Yamaguchi, Japan**Japan
JOURNAL: Japanese Journal of Pharmacology 79 (SUPPL. 1): p99P 1999 1999
MEDIUM: print

CONFERENCE/MEETING: 72nd Annual Meeting of the Japanese Pharmacological Society Sapporo, Japan March 22-25, 1999; 19990322
SPONSOR: Japanese Pharmacological Society
ISSN: 0021-5198
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

7/7/14
DIALOG(R)File 5:Biosis Previews(R)

0011825841 BIOSIS NO.: 199900085501
Sites of regulatory interaction between calcium ATPases and %%%phospholamban%%%
BOOK TITLE: Annals of the New York Academy of Sciences; Cardiac sarcoplasmic reticulum function and regulation of contractility
AUTHOR: MacLennan David H; %%%Kimura Yoshihiro%%%; Toyofuku Toshihiko
BOOK AUTHOR/EDITOR: Johnson R G Jr (Editor); Kranias E G (Editor)
AUTHOR ADDRESS: Banting Best Dep. Med. Res., Univ. Toronto, Charles H. Best Inst., 112 College Street, Toronto, ON M5G 1L6, Canada**Canada
SERIES TITLE: Annals of the New York Academy of Sciences 853 p31-42 1998
MEDIUM: print
BOOK PUBLISHER: New York Academy of Sciences (a), 2 East 63rd Street, New York, New York 10021, USA
CONFERENCE/MEETING: Conference Washington, D.C., USA September 27-30, 1997; 19970927
SPONSOR: New York Academy of Sciences
ISSN: 0077-8923 ISBN: 1-57331-130-8 (paper); 1-57331-129-4 (cloth)
DOCUMENT TYPE: Book; Meeting; Book Chapter; Meeting Paper
RECORD TYPE: Citation
LANGUAGE: English

7/7/15
DIALOG(R)File 5:Biosis Previews(R)

0011502791 BIOSIS NO.: 199800297038
%%%Phospholamban%%% domain Ib mutations influence functional interactions with the Ca2+-ATPase isoform of cardiac sarcoplasmic reticulum
AUTHOR: %%%Kimura Yoshihiro%%%; Asahi Michio; Kurzydlowski Kazimierz; Tada Michihiko; Maclennan David H (Reprint
AUTHOR ADDRESS: Banting and Best Dep. Med. Res., Univ. Toronto, Charles H. Best Inst., 112 College St., Toronto, ON M5G 1L6, Canada**Canada
JOURNAL: Journal of Biological Chemistry 273 (23): p14238-14241 June 5, 1998 1998
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Alanine-scanning mutagenesis of amino acids 21-30, forming cytoplasmic domain 1b in %%%phospholamban%%% (PLN), revealed that mutation to Ala of Asn27, Gln29, and Asn30 results in gain of inhibitory function. In an earlier study (Kimura, Y., Kurzydlowski, K., Tada, M., and MacLennan, D. H. (1997) J. Biol. Chem. 272,15061-15064), gain of function in PLN transmembrane domain II mutants was correlated with pentamer destabilization, leading to proposals that the PLN monomer is the active inhibitory species, that dissociation of the PLN pentamer is one determinant of PLN inhibitory function and that dissociation of the PLN-cardiac sarco(endo)plasmic Ca2+-ATPase isoform (SERCA2a) complex is a second determinant. Because each of the new domain 1b mutants contained a normal ratio of pentamer to monomer in SDS-polyacrylamide gel electrophoresis, gain of function must have resulted from mechanisms other than destabilization of pentameric structure. Evidence that domain 1b and domain II mutants act through different sites and different mechanisms was provided by a monomeric double mutant, N30A/I40A, in which the enhanced inhibitory function of each single mutant was additive.

Evidence for an alteration in stability of the PLN/SERCA2a heterodimer was obtained in a study of double mutant N27A/N34A in which inhibitory function was regained by combining a gain of function, domain Ib mutation with a loss of function domain II mutation. These results support the proposal that PLN inhibition of SERCA2a involves, first, depolymerization of PLN and, second, the formation of inhibitory interactions between monomeric PLN and SERCA2a.

7/7/16
DIALOG(R)File 5:Biosis Previews(R)

0011429138 BIOSIS NO.: 199800223385
%%%Phospholamban%%% domain I/cytochrome b5 transmembrane sequence chimeras do not inhibit SERCA2a
AUTHOR: %%%Kimura Yoshihiro%%%; Asahi Michio; Kurzydlowski Kazimierz; Tada Michihiko; Maclennan David H (Reprint
AUTHOR ADDRESS: Banting Best Dep. Med. Res., Univ. Toronto, Charles H. Best Inst., Toronto, ON M5G 1L6, Canada**Canada
JOURNAL: FEBS Letters 425 (3): p509-512 April 3, 1998 1998
MEDIUM: print
ISSN: 0014-5793
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A series of chimeras between the transmembrane domains of %%%phospholamban%%% (PLN) and cytochrome b5 were coexpressed with the Ca2+-ATPase of cardiac sarcoplasmic reticulum (SERCA2a). The chimeric molecules were not inhibitory, in line with our view that inhibitory PLN/SERCA2a interactions occur in transmembrane sequences, while cytoplasmic interactions regulate the inhibitory interactions in a four-base circuit.

7/7/17
DIALOG(R)File 5:Biosis Previews(R)

0011039962 BIOSIS NO.: 199799674022
Asymmetric distribution of function in the transmembrane domain of %%%phospholamban%%%
AUTHOR: %%%Kimura Yoshihiro%%%; MacLennan David H
AUTHOR ADDRESS: Banting Best Dep. Medical Res., Univ. Toronto, Toronto, ON, Canada**Canada
JOURNAL: Journal of Molecular and Cellular Cardiology 29 (6): pA225 1997 1997
CONFERENCE/MEETING: XIX Annual Meeting of the International Society for Heart Research (American Section) on Cardiovascular Injury, Repair and Adaptation Vancouver, British Columbia July 23-27, 1997; 19970723
ISSN: 0022-2828
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

7/7/18
DIALOG(R)File 5:Biosis Previews(R)

0010970338 BIOSIS NO.: 199799604398
%%%Phospholamban%%% inhibitory function is activated by depolymerization
AUTHOR: %%%Kimura Yoshihiro%%%; Kurzydlowski Kazimierz; Tada Michihiko; Maclennan David H (Reprint
AUTHOR ADDRESS: Charles H. Best Inst., Univ. Toronto, 112 College St., Toronto, ON M5G 1L6, Canada**Canada
JOURNAL: Journal of Biological Chemistry 272 (24): p15061-15064 1997 1997
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% (PLN), a homopentameric, integral membrane protein, reversibly inhibits cardiac sarcoplasmic reticulum Ca-2+-ATPase (SERCA2a) activity through intramembrane interactions. Here, alanine-scanning mutagenesis of the PLN transmembrane sequence was used to identify two functional domains on opposite faces of the transmembrane helix. Mutations in one face diminish inhibitory interactions with transmembrane sequences of SERCA2a, but have relatively little effect on the pentameric state, while mutations in the other face activate inhibitory interactions and enhance monomer formation. Double mutants are monomeric, but loss of inhibitory function is dominant over activation of inhibitory function. These observations support the proposal that the SERCA2a interaction site lies on the helical face which is not involved in pentamer formation. Four highly inhibitory mutants are effectively devoid of pentamer, suggesting that pentameric PLN represents a less active or inactive reservoir that dissociates to provide inhibitory monomeric PLN subunits. A model is presented in which the degree of PLN inhibition of SERCA2a activity is ultimately determined by the concentration of the inhibited PLN monomer cntdot SERCA2a heterodimeric complex. The concentration of this inhibited complex is determined by the dissociation constant for the PLN pentamer (which is mutation-sensitive) and by the dissociation constant for the PLN/SERCA2a heterodimer (which is likely to be mutation-sensitive).

7/7/19
DIALOG(R)File 5:Biosis Previews(R)

0010549040 BIOSIS NO.: 199699183100
%%%Phospholamban%%% regulates the Ca-2+-ATPase through intramembrane interactions
AUTHOR: %%%Kimura Yoshihiro%%%; Kurzydlowski Kazimierz; Tada Michihiko; Maclennan David H (Reprint
AUTHOR ADDRESS: Banting Best Dep. Med. Res., Univ. Toronto, Charles H. Best Inst., 112 College St., Toronto, ON M5G 1L6, Canada**Canada
JOURNAL: Journal of Biological Chemistry 271 (36): p21726-21731 1996 1996
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: There is clear evidence for direct regulatory protein-protein interactions between %%%phospholamban%%% (PLN) and the Ca-2+-ATPase of cardiac sarcoplasmic reticulum (SERCA2a) in cytoplasmic domains, but there is less clear evidence for regulatory interactions in the transmembrane domains of the two proteins. We have now coexpressed SERCA isoforms with the transmembrane sequence of PLN and with epitope-tagged transmembrane sequences of PLN to study intramembrane interactions in the absence of cytoplasmic interactions. Coexpression of the transmembrane sequence of %%%phospholamban%%% (Met-PLN-28-52) with SERCA1a, SERCA2a, and SERCA3 inhibited Ca-2+ transport by lowering apparent Ca-2+ affinity. Addition of the hemagglutinin (HA) epitope to the transmembrane sequence of PLN (HA-PLN-28-52) or deletion of PLN residues 21-29 (PLN-1-20PLN-30-52) "supershifted" apparent Ca-2+ affinity to values lower than those observed with native PLN without uncoupling Ca-2+ transport from ATP hydrolysis. Inhibition by PLN-1-20-PLN-30-52 or by Flag-PLN-28-52 was reversed by PLN antibody or by Flag antibody, demonstrating that inhibition by these constructs is reversible and that the inhibitory constructs are properly oriented in the membrane. These results suggest that PLN modulates the apparent Ca-2+ affinity of SERCA2a through intramembrane interactions, which are disrupted at long range and in concert with disruption of the well characterized cytoplasmic interactions.

7/7/20
DIALOG(R)File 5:Biosis Previews(R)

0010244942 BIOSIS NO.: 199698712775
%%%Phospholamban%%% constructs with deleted or altered cytoplasmic domains are potent inhibitors of the Ca-2+ pump
AUTHOR: %%%Kimura Yoshihiro%%%; MacLennan David H
AUTHOR ADDRESS: Banting Best Dep. Med. Res., Univ. Toronto, Toronto, ON M5G 1L6, Canada**Canada
JOURNAL: Biophysical Journal 70 (2 PART 2): pA139 1996 1996
CONFERENCE/MEETING: 40th Annual Meeting of the Biophysical Society Baltimore, Maryland, USA February 17-21, 1996; 19960217
ISSN: 0006-3495
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

7/7/21
DIALOG(R)File 5:Biosis Previews(R)

0009487224 BIOSIS NO.: 199497508509
Thyroid hormone enhances Ca-2+ pumping activity of the cardiac sarcoplasmic reticulum by increasing Ca-2+ ATPase and decreasing %%%phospholamban%%% expression
AUTHOR: %%%Kimura Yoshihiro%%%; Otsu Kinya; Nishida Kazuhiko; Kuzuya Tsunehiko; Tada Michihiko (Reprint
AUTHOR ADDRESS: Dep. Med. and Pathophysiol., Osaka Univ. Med. Sch., 2-2 Yamadaoka, Suita, Osaka 565, Japan**Japan
JOURNAL: Journal of Molecular and Cellular Cardiology 26 (9): p1145-1154 1994 1994
ISSN: 0022-2828
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Phospholamban%%% is a putative suppressor of the Ca-2+ ATPase of the cardiac sarcoplasmic reticulum. The level of mRNA encoding the Ca-2+ ATPase has been shown to be increased, whereas the %%%phospholamban%%% mRNA level to be decreased in the ventricles obtained from hyperthyroid rabbits (Nagai R, Zarain-Herzberg A, Brandl CJ, Fujii J, Tada M. MacLennan DH, Alpert NR, Periasamy M. (1989) Proc Natl Acad Sci USA 86: 2966-2970). The present study was designed to examine whether these effects of thyroid hormone on the expression of the Ca-2+ ATPase and %%%phospholamban%%% are exerted directly on cardiac myocytes and whether the resultant incoordinate expression of these proteins alters Ca-2+ pumping activity. We studied the levels of %%%phospholamban%%% and Ca-2+ ATPase mRNA in primary isolated neonatal rat myocardial cells incubated with triiodothyronine (T-3) for 3-48 h and the Ca-2+ uptake activity of the microsomes prepared from the cells. Northern blot analysis showed that T-3 decreased %%%phospholamban%%% mRNA levels to about a half of control in 24 h. On the other hand, Ca-2+ ATPase mRNA gradually increased with time. EC-50 for %%%phospholamban%%% mRNA expression was 2.5 times 10-10 M which was approximately 10 times higher than that for the Ca-2+ ATPase. T-3 increased V-max of Ca-2+ uptake with the significant reduction of K-0.5 for Ca-2+ (0.40 +- 0.02 mu-M for control v 0.31 +- 0.02 mu-M for T-3-treated vesicles), indicating that thyroid hormone stimulates Ca-2+ pumping activity not only by increasing the Ca-2+ ATPase but also decreasing %%%phospholamban%%%. These results suggested that %%%phospholamban%%% regulates the Ca-2+ ATPase in dual modes; in short time range, by decreasing the affinity of the Ca-2+ ATPase for Ca-2+ by phosphorylation of %%%phospholamban%%% with cAMP-dependent protein kinase and in long time range, by changing the molecular ratio between the two proteins through the regulation of gene expression.

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DIALOG(R)File 5:Biosis Previews(R)

0014437619 BIOSIS NO.: 200300396049

Targeted inhibition of Ca2+/calmodulin-dependent protein kinase II in cardiac longitudinal sarcoplasmic reticulum results in decreased %%%phospholamban%%% phosphorylation at threonine 17.
AUTHOR: Ji Yong; Li Bailing; %%%Reed Thomas D%%%; Lorenz John N; Kaetzel Marcia A; Dedman John R (Reprint
AUTHOR ADDRESS: Dept. of Genome Science, University of Cincinnati, 2180 E. Galbraith Rd., Cincinnati, OH, 45237, USA**USA
AUTHOR E-MAIL ADDRESS: John.Dedman@uc.edu
JOURNAL: Journal of Biological Chemistry 278 (27): p25063-25071 July 4, 2003 2003
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: To investigate the role of Ca2+/calmodulin-dependent kinase II in cardiac sarcoplasmic reticulum function, transgenic mice were designed and generated to target the expression of a Ca2+/calmodulin-dependent kinase II inhibitory peptide in cardiac longitudinal sarcoplasmic reticulum using a truncated %%%phospholamban%%% transmembrane domain. The expressed inhibitory peptide was highly concentrated in cardiac sarcoplasmic reticulum. This resulted in a 59.7 and 73.6% decrease in %%%phospholamban%%% phosphorylation at threonine 17 under basal and beta-adrenergic stimulated conditions without changing %%%phospholamban%%% phosphorylation at serine 16. Sarcoplasmic reticulum Ca2+ uptake assays showed that the Vmax was decreased by apprx30% although the apparent affinity for Ca2+ was unchanged in heterozygous hearts. The in vivo measurement of cardiac function showed no significant reductions in positive and negative dP/dt, but a moderate 18% decrease in dP/dt40, indicative of isovolumic contractility, and a 26.1% increase in the time constant of relaxation (tau) under basal conditions. The changes in these parameters indicate a moderate cardiac dysfunction in transgenic mice. Although the 3- and 4-month-old transgenic mice displayed no overt signs of cardiac disease, when stressed by gestation and parturition, the 7-month-old female mice develop dilated heart failure, suggesting the important role of Ca2+/calmodulin-dependent kinase II pathway in the development of cardiac disease.

9/7/2
DIALOG(R)File 5:Biosis Previews(R)

0013380867 BIOSIS NO.: 200100552706
Replacement of the muscle-specific sarcoplasmic reticulum Ca2+-ATPase isoform SERCA2a by the nonmuscle SERCA2b homologue causes mild concentric hypertrophy and impairs contraction-relaxation of the heart
AUTHOR: Ver Heyen Mark; Heymans Stephane; Antoons Gudrun; %%%Reed Thomas%%% ; Periasamy Muthu; Awede Bonaventure; Lebacq Jean; Vangheluwe Peter; Dewerchin Mieke; Collen Desire; Sipido Karin; Carmeliet Peter; Wuytack Frank (Reprint
AUTHOR ADDRESS: Laboratory of Physiology, Department of Medicine, KU Leuven, Herestraat 49, Campus Gasthuisberg, B-3000, Leuven, Belgium** Belgium
JOURNAL: Circulation Research 89 (9): p838-846 October 26, 2001 2001
MEDIUM: print
ISSN: 0009-7330
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The cardiac sarco(endo)plasmic reticulum Ca2+-ATPase gene (ATP2A2) encodes the following two different protein isoforms: SERCA2a (muscle-specific) and SERCA2b (ubiquitous). We have investigated whether this isoform specificity is required for normal cardiac function. Gene targeting in mice successfully disrupted the splicing mechanism responsible for generating the SERCA2a isoform. Homozygous SERCA2a-/- mice displayed a complete loss of SERCA2a mRNA and protein resulting in a switch to the SERCA2b isoform. The expression of SERCA2b mRNA and protein in hearts of SERCA2a-/- mice corresponded to only 50% of wild-type SERCA2

levels. Cardiac %%%phospholamban%%% mRNA levels were unaltered in SERCA2a-/- mice, but total %%%phospholamban%%% protein levels increased 2-fold. The transgenic phenotype was characterized by a apprxeq20% increase in embryonic and neonatal mortality (early phenotype), with histopathologic evidence of major cardiac malformations. Adult SERCA2a-/- animals (adult phenotype) showed a reduced spontaneous nocturnal activity and developed a mild compensatory concentric cardiac hypertrophy with impaired cardiac contractility and relaxation, but preserved beta-adrenergic response. Ca2+ uptake levels in SERCA2a-/- cardiac homogenates were reduced by apprxeq50%. In isolated cells, relaxation and Ca2+ removal by the SR were significantly reduced. Comparison of our data with those obtained in mice expressing similar cardiac levels of SERCA2a instead of SERCA2b indicate the importance of the muscle-specific SERCA2a isoform for normal cardiac development and for the cardiac contraction-relaxation cycle.

9/7/3
DIALOG(R)File 5:Biosis Previews(R)

0012556714 BIOSIS NO.: 200000275027
The expression of SR calcium transport ATPase and the Na+/Ca2+ exchanger are antithetically regulated during mouse cardiac development and in hypo/hyperthyroidism
AUTHOR: %%%Reed Thomas D%%%; Babu Gopal J; Ji Yong; Zilberman Alla; Ver Heyen Mark; Wuytack Frank; Periasamy Muthu (Reprint
AUTHOR ADDRESS: Division of Cardiology, Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, 45267-0524, USA**USA
JOURNAL: Journal of Molecular and Cellular Cardiology 32 (3): p453-464 March, 2000 2000
MEDIUM: print
ISSN: 0022-2828
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The mouse has been used extensively for generating transgenic animal models to study cardiovascular disease. Recently, a number of transgenic mouse models have been created to investigate the importance of sarcoplasmic reticulum (SR) Ca2+ transport proteins in cardiac pathophysiology. However, the expression and regulation of cardiac SR Ca2+ ATPase and other Ca2+ transport proteins have not been studied in detail in the mouse. In this study, we used multiplex RNase mapping analysis to determine SERCA2, %%%phospholamban%%% (PLB), and Na+/Ca2+-exchanger (NCX-1) gene expression throughout mouse heart development and in hypo/hyperthyroid animals. Our results demonstrate that the expression of SERCA2 and PLB mRNA increase eight-fold from fetal to adult stages, indicating that SR function increases with heart development. In contrast, the expression of the Na+/Ca2+-exchanger gene is two-fold higher in fetal heart compared to adult. Our study also makes the important observation that in hypothyroidic hearts the NCX-1 mRNA and protein levels were upregulated, whereas the SERCA2 mRNA/protein levels were downregulated. In hyperthyroidic hearts, however, an opposite response was identified. These findings are important and point out that the expression of NCX-1 is regulated antithetically to that of SERCA2 during heart development and in response to alterations in thyroid hormone levels.

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